Vol. 9 May, 1949 No. 2

JOURNAL OF THE FACULTY OF AGRICULTURE KYUSHU UNIVERSITY

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PUBLISHED BY THE FACULTY
FUKUOKA
1949

Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

INVESTIGATIONS ON THE HYMENOPTEROUS PARASITES OF *CEROPLASTES RUBENS* MASKELL IN JAPAN*

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Plates 1–2

INTRODUCTION

Ceroplastes rubens Maskell has been an important pest of various economic plants in Japan for more than fifty years. Since its introduction into Japan (first found in 1897 at Nagasaki), it has spread over practically all provinces of Kyushu and Shikoku and western half of the main island, Honshu. The fact that no less than one hundred and twenty-five species of plants are attacked by this scale in Japan indicates the difficulty of its complete control by the chemical methods. Thus this scale is considered to be the third most important *citrus*-scale in Japan or the second most serious persimmon-pest in Japan, although it is of minor importance in foreign countries. Therefore the introduction of its parasitic Hymenoptera has been attempted during the years 1932 to 1938 from Hawaii and California, as the control of this scale by the introduction of its natural enemies seemed to be most promising. Four parasitic Hymenoptera, viz. *Aneristus ceroplastae* Howard, *Microterys kotinskyi* (Fullaway), *Tomocera californica* Howard and *Scutellista cyanea* Motschulsky, were introduced and liberated at Nagasaki, but without success. Meanwhile, an

* Paper read at the Seventh General Meeting of the Entomological Society of Japan at Kyoto University, Kyoto, and at the Fourteenth Plant Diseases and Pests Control Conference for the Kyushu District at Beppu.

elaborate investigation on the native parasites and predators has been carried out at the Nagasaki Agricultural Experiment Station, and several parasitic Hymenoptera were found. Unfortunately, however, these parasites were proved to be of little importance in checking the scale. On the basis of all available evidences, Professor Tei Ishii concluded in 1940 that the only possible method of control of this scale, therefore, would be the chemical one.

But it must be noted that the survey of the natural enemies of this scale has been performed chiefly at Nagasaki and that the horticulturists must expect some injuries to fruit trees from the application of insecticides in various ways.

In the early summer of 1946, the senior author had the good fortune to rear three species of parasitic Hymenoptera, viz. *Anicetus annulatus* Timberlake, *Microterys speciosus* Ishii and *Coccophagus hawaiiensis* Timberlake, from *Ceroplastes rubens* Maskell at Fukuoka. The percentage of parasitism of the latter two species was very low as previously examined by Dr. Ishii at Nagasaki, while that of the first mentioned species was comparatively high. Therefore, the senior author arrived at the supposition that the data are presented as local evidence of the effectiveness of this parasite in partially controlling the scale, and further recollected the word of Dr. C. P. Clausen, that one phase of biological control which has been somewhat neglected is that of the utilization of native parasites and predators. *Anicetus annulatus* Timberlake was hitherto known as a parasite of five other species of scales, and it is quite possible that the usefulness of this parasite has generally been overlooked.

In 1947, studies were made by the authors chiefly to ascertain the effectiveness of this parasite in Kyushu. Scales were collected from thirty-six localities in both Fukuoka and Oita Prefectures. The collections were made at random in each locality from various host plants, and the scales were examined daily for the emergence of the parasites. Such field collections and the results obtained would have convinced the authors that *Anicetus annulatus* Timberlake was really serving a good purpose in controlling the scales in Fukuoka Prefecture.

Before going further the authors express their hearty gratitude to Professor Teiso Esaki for his kindness rendered in the course of the present study.

Parasites of *Ceroplastes rubens* Maskell reared in Kyushu

Following eight species of parasitic Hymenoptera were reared from *Ceroplastes rubens* throughout the course of this investigation.

Aphelinidae

1. *Aphytis* sp.

16♀♀, Fukuoka, 6. v.—30. v. 1947; 15♀♀, Tsukumi, 24. iv. —25. v. 1947.

2. *Coccophagus hawaiiensis* Timberlake

1921 *Coccophagus lecanii* Nakayama (nec Fitch), Phil. Jour. Sci., 18: 98.
1926 *Coccophagus japonicus* Gahan, Proc. Ent. Soc. Washington, 28: 24.
1931 *Coccophagus hawaiiensis* Compere, Proc. U. S. Nat. Mus., 78, 7: 55.
1932 *Coccophagus hawaiiensis* Ishii, Oyo-Dobuts. Zasshi, 4: 151.
1940 *Coccophagus japonicus* Ishii (nec Compere), Oyo-Dobuts. Zasshi, 12: 123.

2♀♀, Fukuoka, 1946; 5♀♀, Fukuoka, 17. v.—31. v. 1947.

Distribution: Japan (Honshu: Shizuoka, Wakayama, Hiroshima. Kyushu: Fukuoka, Nagasaki, Miyazaki, Kagoshima).

Other host: ? *Ceroplastes ceriferus* (Anderson).

3. *Casca* sp.

3♀♀, Fukuoka, 14. v.—29. v. 1947; 1♀, Tsukumi, 25. iv. 1947.

Encyrtidae

4. *Microterys speciosus* shii

1923 *Microterys speciosus* Ishii, Dept. Agr. & Comm. Japan, Imp. Plant Quar. Sta., Bull., 3: 70.
1928 *Microterys speciosus* Ishii, Bull. Imp. Agr. Exp. Sta., 3: 133.
1932 *Microterys speciosus* Ishii, Oyo-Dobuts. Zasshi, 4: 151.
1940 *Microterys speciosus* Ishii, Oyo-Dobuts. Zasshi, 12: 123.

5♀♀, 1♂, Fukuoka, 26. iv.—4. v. 1947.

Distribution: Japan (Honshu: Shizuoka. Kyushu: Fukuoka, Nagasaki, Kagoshima).

Other host: *Ceroplastes floridensis* Comstock.

5. *Microterys okitsuensis* Compere

1926 *Microterys okitsuensis* Compere, Univ. California Pubns., Ent., 4: 38.
1928 *Microterys okitsuensis* Ishii, Bull. Imp. Agr. Exp. Sta., 3: 137.

3♀♀, 7♂♂, Fukuoka, 30. iv.—25. v. 1947; 1♀, 1♂, Tsukumi, 26. iv.—17. v. 1947.

Distribution: Japan (Honshu: Okitsu. Kyushu: Fukuoka, Nagasaki, Tsukumi).

Other hosts: *Pulvinaria aurantii* Cockerell, *Pulvinaria psidi* Maskell.

6. *Anabrolepis bifasciata* Ishii

1923 *Anabrolepis bifasciata* Ishii, Dept. Agr. & Comm. Japan, Imp. Plant Quar. Sta., Bull., 3: 106.
1928 *Anabrolepis bifasciata* Ishii, Bull. Imp. Agr. Exp. Sta., 3: 148.

1♀, Tsukumi, 14. v. 1947.

Distribution: Japan (Kyushu: Nagasaki, Tsukumi).

7. *Anabrolepis extranea* Timberlake

1920 *Anabrolepis extranea* Timberlake, Proc. Hawai. Ent. Soc., 4: 434.
1928 *Anabrolepis extranea* Ishii, Bull. Imp. Agr. Exp. Sta., 3: 148.

1♀, Tsukumi, 27. iv. 1947.

Distribution: Hawaii, Japan (Honshu: Ozuki. Kyushu: Isahaya, Tsukumi).

Other host: *Pseudaonidia paeoniae* Cockerell.

In 1920 Dr. Timberlake wrote as follows: "this certainly must be an immigrant species, but it is not yet apparent from what part of the world it was derived, although an Oriental origin is suspected."

8. *Anicetus annulatus* Timberlake

1919 *Anicetus annulatus* Timberlake, Proc. Hawai. Ent. Soc., 4: 227.
1920 *Anicetus annulatus* Fullaway, Proc. Hawai. Ent. Soc., 4: 242.
1928 *Anicetus annulatus* Ishii, Bull. Agr. Exp. Sta., 3: 149.

40♀♀, 25♂♂, Fukuoka, 1946: 1173♀♀, 722♂♂, Fukuoka, 1947: 4♂♂, Tsukumi, 25. iv.–4. v. 1947: 1♀, Beppu, 1947.

Distribution: California, Hawaii, Japan (Honshu: Yokohama. Kyushu: Fukuoka, Beppu, Nagasaki, Tsukumi).

Other hosts: *Coccus hesperidum* Linné, *Coccus pseudomagnoliarum* (Kuwana), *Eucalymnatus tesselatus* Signoret, *Pulvinaria* sp., *Ceroplastes ceriferus* Anderson (new host, Beppu and Kashii), *Saissetia hemisphaerica* (Targioni).

Among these parasites *Microterys speciosus* and *Coccophagus hawaiiensis* are the hitherto known natural enemies of *Ceroplastes rubens*. To determine the order of importance of these parasites, the results of the 1947 survey are set forth in Table 1.

Table 1. Percentage of parasites of *Ceroplastes rubens* observed in Kyushu in 1947.

Parasites	No. of individuals reared	Percentage
Anicetus annulatus	1870	97.04
Aphytis sp.	31	1.52
Microterys okitsuensis	12	0.59
Microterys speciosus	6	0.29
Coccophagus hawaiiensis	5	0.24
Casca sp.	4	0.19
Anabrolepis bifasciata	1	0.06
Anabrolepis extranea	1	0.06

From this table it is apparent that *Anicetus annulatus* is by far the most important parasite of *Ceroplastes rubens* and that seven other species are almost ineffective in reducing the scale populations. It is an interesting fact that the two species, *Microterys speciosus* and *Coccophagus hawaiiensis*, were found only in small numbers and have never made any increase in the level of effectiveness during the past twenty years. As to the economic status of the former species Dr. Ishii wrote in 1923 as follows: " it has already been noted that the parasite prefers to deposit eggs in full-grown female hosts, and that the latter is not killed until the greater proportion of their eggs are laid. Furthermore, the parasite usually develops only one generation per year in this host, and the percentage parasitized is very low, averaging about 3%. ... For this reason given above, and substantiated by observations and extensive counts in the field, it may be said that the parasite has very little or no effect in checking the scale." As to *Coccophagus hawaiiensis* he further wrote as follows: " ... comparatively large numbers of pupae and adults were found dead in the host scale, this probably being due to premature death and drying out of the hosts, these consequently becoming so hard that the adult is unable to effect emergence. The adults of all generations prefer small immature scales in which to deposit their eggs, and particularly the adults of the first, third and fourth generations oviposit largely in such scales whose growth has not been vigorous. The above facts, together with the relating low percentage of parasitism, show that *C. hawaiiensis* (*lecanii*) is of very little value as a means of checking the increase of *Ceroplastes*

rubens." The authors' investigation indicates that the parasitism of these two parasites is much lower than that observed by Dr. Ishii. As economic entomologists, the authors feel no need to advance their knowledge of these seven species. Nevertheless, from the theoretical standpoint, the authors are extremely interested in the fact that the species of the Genera *Aphytis*, *Casca* and *Anabrolepis* were hitherto unrecorded from the scale insects of the Genus *Ceroplastes*.

Percentage of Parasitism of *Ceroplastes rubens* by Hymenopterous Parasites observed in Kyushu

In the course of the present investigation, the percentage of parasitism was calculated by the following formula: $\frac{\text{Number of adult parasites emerged}}{\text{Number of scales}} \times 100$. The value, however, does not represent the real number of scales which were practically killed by the parasitic larvae. The larvae of the parasites which could not complete metamorphosis in the course of the postembryonic developmental period owing to some physiological reasons were not calculated in the present investigation. Consequently the value derived from the formula does not indicate an actual percentage of parasitism. Thus it is claimed that the actual value of parasitism must be estimated much higher. The results are summarized in Table 2.

Table 2. Parasitism of *Ceroplastes rubens* by Hymenopterous parasites in different localities in Kyushu observed in 1947.

Host plants of *Ceroplastes rubens*	Localities*	No. of *C. rubens*	No. of parasites emerged*	Percentage of parasitism
Thea sinensis	Beppu, Oita Pref.**	114	A1♀	0.87
Citrus Unshu	Hikonouchi, Ts	252	0	0.00
Citrus Unshu	Nishinouchi, Ts	470	0	0.00
Citrus Unshu	Nakata, Ts	78	0	0.00
Citrus Unshu	Nishinouchi, Ts	1589	D1♀, F1♀	0.13
Citrus Unshu	Nishinouchi, Ts	294	0	0.00
Citrus Unshu	Kataura, Ts	165	0	0.00
Citrus Unshu	Shimoaoe, Ts	286	0	0.00
Eurya japonica	Nakata, Ts	3176	A1♀	0.03

Eurya japonica	Nakata, Ts	396	A1♂, G14♀	3.78
Thea sinensis	Nishinouchi, Ts	170	0	0.00
Thea sinensis	Hikonouchi, Ts	783	A2♂, E1♀, H1♀	0.51
Thea sinensis	Nakata, Ts	591	0	0.00
Thea sinensis	Iwaya, Ts	746	G1♀	0.13
Sakakia ochracea	Miyamoto, Ts	164	0	0.00
Camellia Sasanqua	Miyamoto, Ts	53	0	0.00
Diospyros Kaki	Nishinouchi, Ts	1764	0	0.00
Camellia japonica	Hikonouchi, Ts	58	0	0.00
Cinnamomum camphora	Miyamoto, Ts	114	D1♂	0.87
Laurus nobilis	Nakata, Ts	619	0	0.00
Fortunella japonica	Miyajidake, Fu	40	A5♀9♂	35.00
Ilex latifolia	University campus, Fu	226	A65♀43♂, C3♀	49.11
Machilus Thunbergii	University campus, Fu	171	A39♀19♂	33.91
Cinnamomum Loureirii	University campus, Fu	604	A100♀91♂	31.62
Ilex rotunda	University campus, Fu	677	A8♀2♂	1.47
Thea sinensis	University campus, Fu	567	A89♀55♂	25.39
Diospyros Kaki	Sasaguri, Fu	1667	A141♀85♂, G14♀	14.39
Citrus Unshu	Tachibana, Fu	1213	A128♀41♂, D3♀6♂	14.67
Illicium anisatum	University campus, Fu	179	A59♀27♂	48.04
Eurya japonica	Harumachi, Fu	1720	A138♀50♂, B1♀, D1♂, G2♀, H1♀	11.22
Sasakia ochracea	Sasaguri, Fu	962	A79♀64♂, B1♀, H1♀	15.07
Ilex dimorphophylla	University campus, Fu	560	A12♀17♂	5.17
Ilex integra	University campus, Fu	1307	A138♀76♂	16.37
Sasakia ochracea	Sasaguri, Fu	368	A23♀20♂, B3♀, C2♀1♂	13.31
Camellia japonica	Harumachi, Fu	1240	A104♀124♂	18.38
Laurus nobilis	University campus, Fu	1805	A45♀69♂	6.31

* Ts : Tsukumi, Oita Prefecture. Be : Fu : Fukuoka Prefecture.

A : *Anicetus annulatus.* B : *Coccophagus hawaiiensis.* C : *Microterys speciosus.* D : *Microterys okitsuensis.* E : *Anabrolepis extranea.* F : *Anabrolepis bifasciata.* G : *Aphytis* sp. H : *Casca* sp.

** Kindly collected by Mr. Tsuneo Torikata to whom the authors express their hearty thanks.

As seen from the table given above, marked differences in parasitism could be found in different localities. The parasitism at Beppu district was only 0.87 per cent and at Tsukumi district averaged only 0.27 per cent; whereas at Fukuoka district it averaged 21.1 per cent. Furthermore, the results show a high degree of parasitism in some places in Fukuoka district. The failure to find *Anicetus annulatus* in any appreciable numbers in Oita Prefecture (Beppu and Tsukumi) in the present investiga-

tion seems to be attributed to the later invasion of *Ceroplastes rubens* into that district.

The material from the University campus was collected mainly in the Botanical garden of the Faculty of Agriculture, Kyushu University. The following host plants are planted close to each other in a very limited area in the garden, and the comparison of the parasitism of *Ceroplastes rubens* on these plants seems to throw some light upon the habitat of the important parasite, *Anicetus annulatus*.

Table 3. Actual parasitism of *Anicetus annulatus* on *Ceroplastes rubens* on different host plants cultivated in a very limited area in the Botanical garden of the Faculty of Agriculture, Kyushu University.

Groups	Hosts	Host plants	Percentage of practical parasitism
A	*Ceroplastes rubens*	*Ilex latifolia*	over 49.1
	C. rubens	*Machilus Thunbergii*	over 33.9
	C. rubens	*Cinnamomum Loureirii*	over 31.6
	C. rubens	*Thea sinensis*	over 25.4
	C. rubens	*Illicium anisatum*	over 48.0
B	*C. rubens*	*Ilex dimorphophylla*	over 5.2
	C. rubens	*Ilex integra*	over 16.3
	C. rubens	*Laurus nobilis*	over 6.3

As can be seen in the table given above, *Anicetus annulatus* killed a much higher percentage of the population of *Ceroplastes rubens* on the plants of the group A than that of the group B. The highest case of parasitism observed amounted to over 50 per cent. In contrast, scales that were on the group B plants lived without appreciable damage from the parasites. The fact may be regarded as a highly important phenomenon. The questions which now arise for discussion are: (1) Why were the scales on the "A" plants more highly parasitized than those on the "B" plants? and (2) What is the factor which causes such close correlation between the activity of the parasites and the microclima of their habitat. First of all, it must be considered that the plants of the group B have much denser branches and foliage in a unit space than those of the group A. These conditions would affect the microclima on the plants in comparatively high degree. Consequently each of the groups A and B would offer some different

ecological environment to these parasites. Thus *Anicetus annulatus* seemed to prefer the environment of the plants of the group A to those of B. The *Citrus*-trees may also be regarded as a representative of the group A. Similarly the fact that the plants of the group B are generally more densely covered with the sooty mold fungus, which grows on the honey dew given off by *Ceroplastes rubens*, may be of equal significance. In this case the surface of the infested plants appears to be densely covered with black dust. In 1941, Dr. S. F. Flanders published the following very interesting opinion concerning a dusty environment to insects. "A dusty environment is unfavorable to many species of insects. ... The susceptibility of the Hymenoptera to dust is correlated with specialized structures for removing dust particles from their food or from the surface of their bodies. ... The parasitic hymenopteron may pass the debris, removed from the antennae, mouth parts and other parts of the body, backward by means of each pair of legs. ... When small species of parasitic Hymenoptera, such as *Trichogramma*, come in contact with an excessive amount of dust, their movements lack coordination and they "wallo" helplessly. ... The parasites of pests not only may be more susceptible to dusts but they may be more uniformly affected than their hosts since they are largely hymenopterous or dipterous and consequently are more uniform in structure and in their feeding habits." Such a condition seems worthy of consideration. And in this connection it would be suggested that the sooty mold fungus on the infested plants does not directly affect *Anicetus annulatus* but seems to afford an unfavourable environment to this parasite. Invariably there may be numerous natural factors which tend to reduce the populations of the scales. Perhaps the most important of these are adverse climate, diseases, predacious enemies and insect parasites. But in Japan neither climatic factors nor diseases killed a conspicuous number of *Ceroplastes rubens* during the period when these observations were made. *Anicetus annulatus*, on the other hand, destroyed a large number of this scale, particulary in Fukuoka Prefecture. It was roughly estimated that more than fifty per cent of the scales were destroyed by this parasite. At any rate, a remarkable fact connected with this introduced scale is the slowness with which native parasites have transferred to the various stages of the new host. It must be noted that only

in recent years *Anicetus annulatus* has become the dominant parasite of the scales in many places in N. Kyushu. In this connection Prof. Emer. Y. Tanaka* informed the senior author that the population of *Ceroplastes rubens* in his orchard has reduced prominently in recent years. Mr. I. Tateishi* of the Saga Agricultural Experiment Station has also experienced similar cases in some parts of Fukuoka and Saga Prefectures where the number of the scales was checked by the parasitic Hymenoptera. The authors think that all this evidence may represent the effectiveness of *Anicetus annulatus*. This evidence points to the possible importance of distributing the native parasite, *Anicetus annulatus*, from Fukuoka Prefecture to another, even though the species is widespread, as Dr. Clausen (1936) has already suggested.

Emergence of the Adult Parasites

The emergence of such adult parasites as *Aphytis* sp., *Coccophagus hawaiiensis*, *Casca* sp., *Microterys speciosus*, *M. okitsuensis*, *Anabrolepis bifasciata* and *A. extranea* lasted from the end of April to that of May in 1947. In Tsukumi district *Anicetus annulatus* was first observed at the end of April. The dates of the emergence of the adult of this parasite observed in the laboratory of the Kyushu University are summarized in the following tables.

Table 4. Emergence of the adult *Anicetus annulatus* in Fukuoka (Observed in the laboratory).

Date	4. vi.	5. vi.	6. vi.	7.–12. vi.	13. vi.	14.–15. vi.
♀	2	1	1	137	17	209
♂	3	5	4	159	29	222
Total	5	6	5	296	46	431

Date	17. vi.	18. vi.	19. vi.	20. vi.	21. vi.	22. vi.	23. vi.	24. vi.
♀	101	105	151	141	100	46	61	36
♂	84	52	91	65	29	10	21	9
Total	185	157	242	206	129	56	82	45

* To these gentlemen the authors express their thanks for their kind information on the problem.

Date	25. vi.	26. vi.	27. vi.	28. vi.	29. vi.	30. vi.	1. vii.	2. vii.
♀	20	15	9	6	3	0	1	2
♂	1	0	1	0	1	0	1	0
Total	21	15	10	6	4	0	2	2

Table 5. Sex-ratio of the adult *Anicetus annulatus*.

Date	No. of females	No. of males	Sex-ratio in per cent
4. vi.–6. vi.	4	14	22.2
7. vi.–12. vi.	137	159	46.2
13. vi.–17. vi.	318	338	48.5
18. vi.–22. vi.	533	247	68.3
23. vi.–27. vi.	142	34	80.7
28. vi.–2. vii.	12	2	85.7
Total number	1146	794	

From these tables it is apparent that the emergence of the adult *Anicetus annulatus* was at its height during the middle of June when the sex-ratio is about 50 per cent. Next, the time of the emergence of the adult in each day was observed in the laboratory.

Table 6. Time of the emergence of the adult *Anicetus annulatus* in Fukuoka (Observed in the laboratory).

Date	19. vi.		24. vi.		25. vi.		26. vi.		Total in per cent		Average in per cent
Weather	Fine		Rainy		Fine		Fine				
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
7:00– 8:00	0	11	1	1	1	0	1	0	0.49	11.37	3.88
8:00– 9:00	30	17	1	2	5	1	10	0	17.45	19.58	18.12
9:00–10:00	41	13	7	5	8	0	1	0	28.30	15.46	24.27
10:00–11:00	39	8	13	1	4	0	1	0	24.05	13.40	20.71
11:00–12:00	11	8	12	0	0	1	1	0	11.79	9.27	11.00
12:00–16:00	22	28	2	0	0	0	1	0	11.92	30.95	22.02
16:00– 7:30	0	0	0	0	0	0	0	0	0.00	0.00	0.00

From this table the following facts may be derived. The emergence of the adult begins before seven in the morning, reaches its height during the hours nine to eleven and ceases about four o'clock in the afternoon. More than eighty per cent of the adult emerges before noon, and no adult emerges during the dark hours.

Oviposition of *Anicetus annulatus* and the Size of the Scales

The oviposition behavior of the female of *Anicetus annulatus* was observed very easily under the binocular microscope. When the female is ready to oviposit, she mounts on the back of a scale and examines it carefully with the tips or the underside of the antennae while walking back and forth, always orientating her body-axis almost parallel to the longitudinal axis of the scale. If the scale is in satisfactory condition, she stands on tip-toe, and raises the anterior portion of the body, but lowers the tip of the abdomen until it is close to the protective wax covering of the scale. The ovipositor pierces the protective covering of wax of the host. This piercing procedure may require ten minutes or as much as fifteen minutes. The oviposition may require only a few second. Meanwhile the antennae are almost motionless and pointed downward. Sometimes the host scale exserts its anal tube as the female parasite stabs it with the ovipositor during oviposition, an indication that the scale possibly feels and is in pain during the procedure as already known in many cases. In one instance, the honey dew excrected by the anal tube of the scale became attached to the face of the ovipositing female parasite, but the parasite neither paid any attention to it nor fed on it. *Anicetus annulatus* does not distinguish between scales already oviposited on and those not oviposited on. It is not uncommon to find supernumerary eggs on the same hosts laid by different parasites or by parasites that returned to attack the same host. If the scale is large, she inserts the ovipositor into the scale in full length. If the scale is small, only the apical portion of the ovipositor is inserted. The measurements of 1558 scales from which *Anicetus annulatus* emerged are summarized as follows:

Table 7. Measurements of the protective covering of wax of *Ceroplastes rubens*.

	Minimum length	Maximum length	Average length
Longitudinal axis	1.0 mm.	4.0 mm.	2.48 mm.
Transverse axis	1.0 mm.	4.0 mm.	2.28 mm.

So far as the authors' observations goes, there are no sufficient data and observations available to substantiate the fact that a larger parasite has a tendency to select a larger scale.

Longevity of the Adult Parasites

Generally it is almost impossible to ascertain the longevity of the parasitic Hymenoptera in the field, but it is apparent that they would live much longer in the field than in confinement in glass tubes. As to the longevity of *Anicetus annulatus* Dr. Ishii (1923) gave a single record of 52 days in the female. The authors' investigations on the longevity of the same species in confinement in glass tubes in the laboratory are seen in the following tables.

Table 8. Longevity of the adult *Anicetus annulatus* that were given no food.

Longevity in days		2	3	4	5	6	10	Average
No. of individuals	♀	4	7	9	3	1	2	4.07 days
	♂	1	13	3	4	0	0	3.47 days
	total	5	20	12	7	1	2	

Table 9. Longevity of the adult *Anicetus annulatus* that were fed with water.

Longevity in days		3	4	5	6	7	8	Average
No. of individuals	♀	1	4	8	3	2	1	5.21 days
	♂	1	3	2	4	1	0	5.09 days
	total	2	7	10	7	3	1	

Table 10. Longevity of the adult *Anicetus annulatus* that were fed with honey dew dropped off by the Aphididae.

Longevity in days		3	4	7	8	9	11	14	17	18
No. of individuals	♀	0	1	1	0	3	0	0	1	3
	♂	1	0	0	1	0	1	1	0	1
	total	1	1	1	1	3	1	1	1	4

Longevity in days		20	21	24	26	31	37	38	Average
No. of individuals	♀	1	2	0	0	1	3	1	20.64 days
	♂	1	0	4	1	0	0	0	17.81 days
	total	2	2	4	1	1	3	1	

Table 11. Longevity of the adult *Anicetus annulatus* that were fed with diluted honey.

Longevity in days		11	13	14	16	17	27	30
No. of individuals	♀	0	0	1	1	1	1	0
	♂	1	1	0	0	0	0	1
	total	1	1	1	1	1	1	1

Longevity in days		32	35	36	37	39	Average
No. of individuals	♀	1	1	1	1	2	29.20 days
	♂	0	0	0	0	0	18.00 days
	total	1	1	1	1	2	

Table 12. Longevity of the adult *Anicetus annulatus* that were fed with concentrated honey.

Longevity in days		8	17	21	25	26	28	29
No. of individuals	♀	0	1	0	3	1	3	7
	♂	1	1	1	2	1	1	0
	total	1	2	1	5	2	4	7

Longevity in days		30	31	32	33	Average
No. of individuals	♀	3	1	1	1	16.90 days
	♂	0	0	0	0	21.42 days
	total	3	1	1	1	

All the material of the parasites used in these experiments were 0 to 24 hours old. From the tables given above the following facts may be derived. The longevity of the adult parasites fed with water could not be greatly prolonged as compared with those fed nothing whatsoever. The life of the adult parasites could be greatly prolonged by such a diet as honey dew dropped off by the Aphididae (about five times as long as those given no food). The individuals that were furnished with honey showed much longer adult life (sometimes about seven times as long as those without food). But the authors were not be able to detect any definite difference in the adult life fed with honey dew, diluted honey or concentrated honey. The longevity of the adult life of the other parasites that were fed with no food are summarized in the following table.

Table 13. Longevity of the adult life of seven other parasites that were fed with no food.

Aphytis sp.	♀	Average	4.84 days
Microterys okitsuensis	♀		4.40
	♂		6.00
Microterys speciosus	♀		3.66
	♂		3.60
Coccophagus hawaiiensis	♀		5.50
Casca sp.	♀		3.50
Anabrolepis bifasciata	♀		3.00
Anabrolepis extranea	♀		3.00

Conclusion

It is a remarkable fact that during the past few years there has been a progressive decrease in the population of *Ceroplastes rubens* Maskell in N. Kyushu. A thorough analysis and survey in the infested areas of N. Kyushu definitely indicate that the increase of natural enemies is now having considerable influence on the scale population. As many as eight species of Hymenopterous parasites were recognized. Among them an Encyrtid, *Anicetus annulatus* Timberlake, especially offers possibility of reducing scale populations. At present an attempt to liberate this parasite in other parts of Japan where the percentage of parasitism of the scales by the natural enemies is very low is desirable.

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Appendix: List of known Hymenopterous parasites of the scales of the Genus *Ceroplastes*.

(This list may not be complete but contains all the species to which the senior author can refer at present)

Scales	Parasites	Localities	References
actiniformis Green	*Aneristus ceroplastae* Howard	Ceylon	Howard, Ashmead (1896), Gahan (1924, 1925), Smith, Compere (1928), Compere (1936)
africanus Green	*Anicetus africanus* Girault	N. Africa	Mercet (1925)
	Cheiloneurus angustifrons Compere	N. Africa	Compere (1938)
	Trichomasthus tenuicornis Mercet	N. Africa	Mercet (1925)
argentinus Brèthes	*Prorhopoideus baezi* Brèthes	Argentine	Brèthes (1921)
bergi Cockerell	*Cerapterocerus bonariensis* Brèthes	Argentine	Brèthes (1922)
ceriferus (Anderson)	*Anicetus ceroplastis* Ishii	Japan	Ishii (1928)
	Cheiloneurus ceroplastis Ishii	Japan	Ishii (1928)
	Eusemion sp.	China	Silvestri (1929)
chinensis Nietner	*Scutellista* sp.	Italy	Silvestri (1919)

	Tomocera californica Howard	Spain	Mercet (1924), Smith, Compere (1928)
cirripediformis (Comstock)	*Aneristus ceroplastae* Howard	Porto Rico	Dozier (1925), Compere (1936)
	Aphycus mexicanus Howard	U. S. A.	Howard (1898)
	Plagiomerus cyaneus Ashmead	Porto Rico	Dozier (1925)
destructor Newstead	*Anicetus parvus* Compere	C. Africa	Compere (1937)
	Bothriophryne ceroplastae Compere	C. Africa	Compere (1937)
	Coccidoxenus ugandensis Compere	C. Africa	Compere (1937)
	Coccophagus amblydon Compere	C. Africa	Compere (1937)
	Coccophagus clavellatus Compere	S. Africa	Compere (1931)
	Coccophagus flaviceps Compere	S. Africa	Compere (1931)
	Diversinervus elegans Silvestri	C. Africa	Gurney (1936)
	Euxanthellus sp.	S. Africa	Compere (1936)
	Scutellista cyanea Motschulsky	C. Africa	Gurney (1936),
		Australia	Gurney (1936)
dozieri Cockerell	*Aneristus ceroplastae* Howard	Haiti, Porto Rico	Compere (1936)
	Marietta ceroplastae Howard	Haiti, Porto Rico	Compere (1936)
euphorbiae Cockerell	*Aneristus ceroplastae* Howard	Jamaica	Gahan (1924, 1925), Smith, Compere (1928), Compere (1936)
floridensis Comstock	*Microterys clauseni* Compere	Japan	Compere (1926), Ishii (1928, 1932)
	Microterys speciosus Ishii	Japan	Ishii (1928, 1932)
	Encyrtid sp.	China	Silvestri (1929)
galeatus Newstead	*Eurytoma galeati* Girault	C. Africa	Girault (1916), Gowdey (1917)
	Neomphaloidella ceroplastae Girault	C. Africa	Girault (1916), Gowdey (1917)
	Scutellista cyanea Motschulsky	C. Africa	Girault (1916), Gowdey (1917)
giganteus Dozier	*Homalopoda cristata* Howard	Haiti	Dozier (1937)
	Prococcophagus hispaniolae (Dozier)	Haiti	Compere (1936)
mimosae	*Scutellista gigantea* Berlese	E. Africa	Berlese (1917)
rubens Maskell	*Anabrolepis bifasciata* Ishii	Japan	Yasumatsu, Tachikawa
	Anabrolepis extranea Timberlake	Japan	Yasumatsu, Tachikawa
	Aneristus ceroplastae Howard	Hawaii	Timberlake (1918), Fullaway (1919)
		Fiji	Simmonds (1936)
	Anicetus annulatus Timberlake	Japan	Yasumatsu, Tachikawa
	Aphytis sp.	Japan	Yasumatsu, Tachikawa
	Casca sp.	Japan	Yasumatsu, Tachikawa
	Ceraptероceroides japonicus Ashmead	Japan	Ishii (1940)

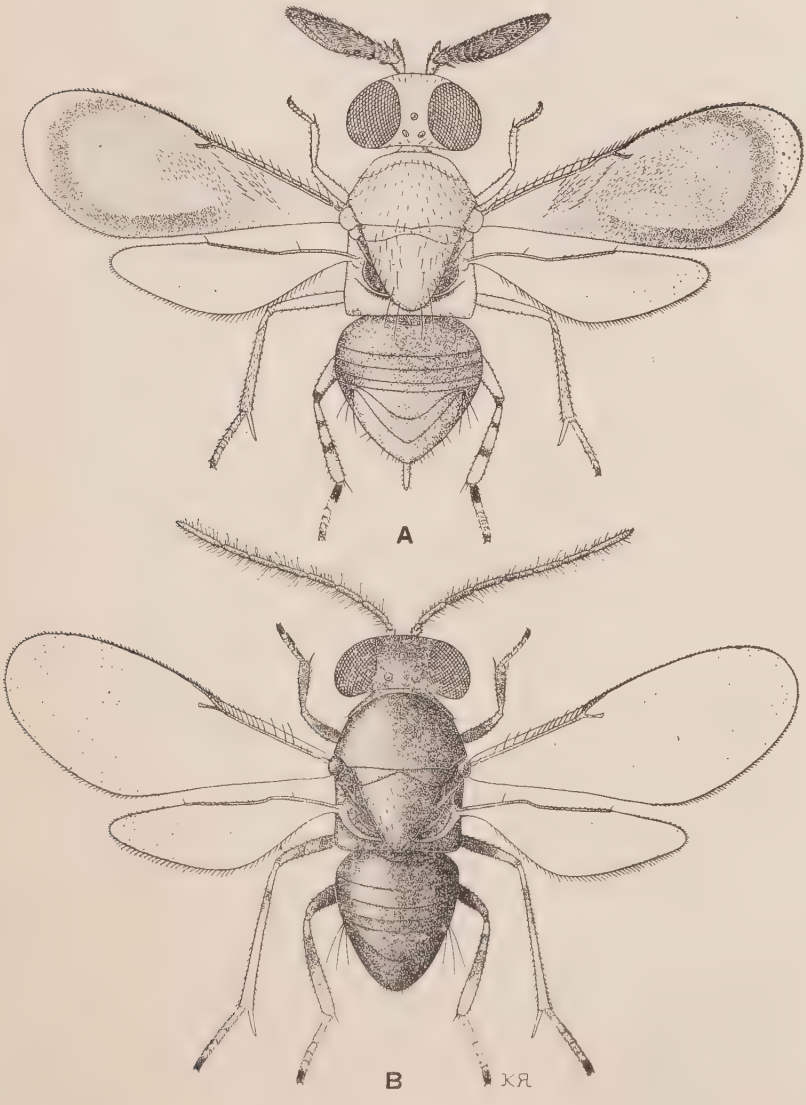
	Cheiloneurus ceroplastis Ishii	Japan	Ishii (1923, 1928, 1932, 1940), Kaburaki (1934)
	Coccophagus hawaiiensis Timberlake	Japan	Ishii (1923, 1932, 1940), Kaburaki (1940), Yasumatsu, Tachikawa
	Eupelmus sp.	Japan	Ishii (1932)
	Eusemion sp.	China	Silvestri (1929)
	Marietta sp.	Japan	Ishii (1940)
	Microterys kotinskyi (Fullaway)	Hawaii	Fullaway (1918)
	Microterys okitsuensis Compere	Japan	Yasumatsu, Tachikawa
	Microterys speciosus	Japan	Ishii (1923, 1928, 1932, 1940), Kaburaki (1934), Yasumatsu, Tachikawa
	Physcus atrithorax Girault	Australia	Girault (1939)
	Quaylea whitieri (Girault)	Hawaii	Ishii (1940)
	Tomocera californica Howard	Hawaii	Fullaway (1919), Smith, Compere (1928)
	Tomocera ceroplastis Perkins	Hawaii	Fullaway (1919)
rusci (Linné)	*Coccophagus howardi* Masi	Europe	Silvestri (1919)
	Coccophagus lecanii (Fitch)	U. S. A.	Smith, Compere (1928)
	Coccophagus scutellaris Dalman	Europe	Silvestri (1919)
	Eusemion italicum Masi	Italy	Masi (1917)
	Marietta caridei (Brèthes)	Brazil	Brèthes (1920)
	Scutellista cyanea Motschulsky	N. Africa	Picard (1914), Delassus (1924)
		S. France	Balachowsky (1927, 1930)
		Italy	Smith, Compere (1928), Widiez (1932)
	Tetrastichus gemani Brèthes	Brazil	Brèthes (1920)
	Tomocera californica Howard	Spain	Mercet (1924), Smith, Compere (1928)
vuilleti Marchal	*Coccidoxenus coelops* Waterston	C. W. Africa	Waterston (1917)
sp.	*Aneristus youngi* Girault	U. S. A.	Compere (1936)
sp.	*Aphycus mexicanus* Howard	Mexico	Howard (1898)
sp.	*Asteropaeus primus* Howard	Mexico	Howard (1898)
sp.	*Bothriophryne ceroplastae* Compere	C. Africa	Compere (1937)
sp.	*Bothriophryne dispar* Compere	C. Africa	Compere (1939)
sp.	*Bothriophryne fuscicornis* Compere	S. Africa	Compere (1939)
sp.	*Bothriophryne purpurascens* Compere	S. Africa	Compere (1939)
sp.	*Ceraptocerus inutilis* Compere	C. Africa	Compere (1937)
sp.	*Coccophagus atratus* Compere	S. Africa	Compere (1926)
sp.	*Coccophagus ispingoensis* Compere	C. Africa	Compere (1937)
sp.	*Coccophagus malthusi* Girault	S. Africa	Compere (1926)
sp.	*Coccophagus margaritatus* Compere	S. Africa	Compere (1931)
sp.	*Coccophagus nubes* Compere	S. Africa	Smith, Compere (1928)

sp.	*Diversinervus elegans* Silvestri	C. Africa	Compere (1937)
sp.	*Eupelmus coccidivorus* Gahan	Panama	Gahan (1924)
sp.	*Lecaniobius capitatus* Gahan	Panama	Gahan (1924)
sp.	*Marietta mexicana* (Howard)	U. S. A.	Howard (1895), Smith, Compere (1928)
		Mexico	Compere (1936)
sp.	*Metaphycus ferrieri* Compere	C. Africa	Compere (1940)
sp.	*Metaphycus lineascapus* Compere	S. Africa	Compere (1940)
sp.	*Microterys elegans* Blanchard	Argentine	Blanchard (1940)
sp.	*Microterys umbrinus* Compere	C. Africa	Compere (1939)
sp.	*Paraceraptrocerus africanus* Girault	S. Africa	Girault (1920)
sp	*Tetrastichodes xenocles* Walker	Chili	Brèthes (1916)

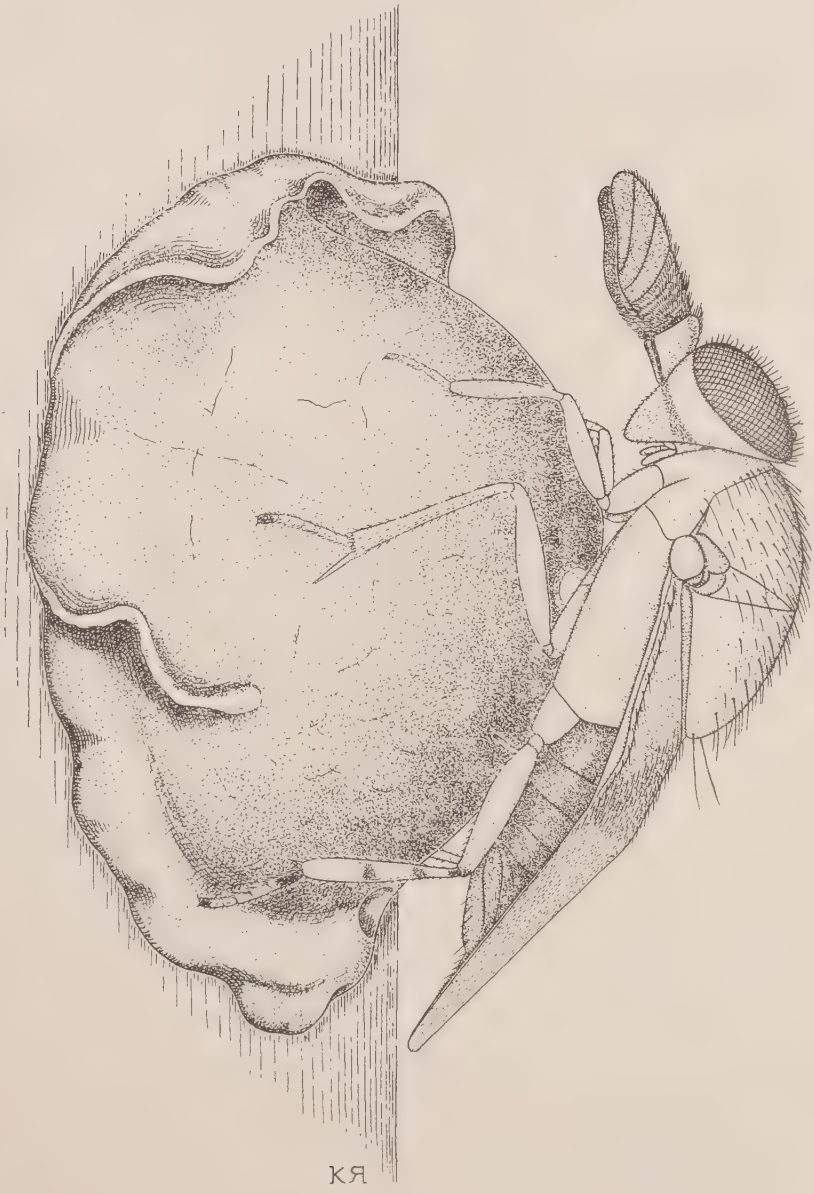
Explanation of the Plates

Plate 1. *Anicetus annulatus* Timberlake, adult.
A: Female
B: Male

Plate 2. *Anicetus annulatus* Timberlake ovipositing in the body of *Ceroplastes rubens* Maskell.



Anicetus annulatus Timberlake



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Anicetus annulatus Timberlake

Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

REARING OF FLEA LARVAE ON VARIOUS DIETS*

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As regards the rearing of fleas, a considerable amount of general information has been accumulated during the past 250 years. But knowledge is scarce particularly about the kind of food used by flea larvae. The following is a review of diets of flea larvae which were used by various authors.

Cestone, J., 1699: the bran-like substance which sticks in the combs when puppies are combed to take out the fleas.

Strickland, C., 1913: refuse from rat cages consisting mainly of dried grain, excreta, gravel, straw, etc. ("rubbish").

Bacot, A. W., 1914: the faeces of the adult flea or anal discharges.

Bacot, A. W. and W. G. Ridewood, 1914: organic matter in the lair of the host, on the dust that collects on the ground in its proximity and the excreta of the adult flea or particles of dried blood.

Bishop, F. C., 1915: partly of blood voided by the adult and partly of particles of animal or vegetable origin.

Lyon, H., 1915: floor sweepings and dried blood.

Illingworth, J. F., 1915: dried blood particles in the dust and the excreta of the adult flea.

Sikes, E. K., 1930: dried blood (human or rabbit) ground up finely.

Sikes, E. K., 1931: dried blood, nest material, flea faeces, albumen.

* Published with the aid of a grant from the Ministry of Education in Japan.

Leeson, H. S., 1932: finely ground dried ox-liver.

Hopkins, G. H. E., 1935: unsterilised bran or maize meal with a pinch of dried ox-blood.

Yasumatsu, K., 1946: dust under the Japanese "Tatami".

The foregoing papers present considerable information on diets of flea larvae; but only Sikes' (1931) work studies the effect of various diets upon the growth of flea larvae. The chief object of the present experiments was to make a preliminary study of the growth-rate of flea larvae on various diets in order to determine the more convenient or economic diets which may be used easily in rearing experiments.

I should like to thank Professor Teiso Esaki, of the Kyushu University, for his kind help and advice during the course of the present experiments, and Doctor Joseph Bequaert, of the Museum of Comparative Zoölogy of Harvard College, for his criticism and and kindness in reading through this manuscript.

Material and Method

The fleas, *Ceratophyllus cheopis* Rothschild, were bred in the laboratory on a rat. The rat was combed carefully and given sterilised bedding, before being provided with identified specimens of *C. cheopis*. Samples of adult fleas were taken frequently from the bed of the rat and identified to make sure that the culture remained pure. Pregnant female fleas were taken from the bed and put in a small glass cylinder placed on black paper. The fleas were left in this for 24 hours, and then returned to the rat, a supply of eggs having been laid in the meantime. The eggs were easy to see and count on black paper. From these eggs 0 to 24 hours old flea larvae were easily collected. In all cases 0 to 24 hours old unfed larvae were used in the present experiments. The flea larvae were bred in glass bottles which were previously provided with sawdust and the following diets, and only those which reached adult stage were counted. The experiments were carried out at room temperature and humidity, but both seemed to make little difference to the survival of the larvae, only affecting the rate of development so far as the present experiments were concerned.

1. Flour of miscellaneous cereals and beans (A).
2. Excrements of silk-worms, dried and powdered (B).
3. Silk-worm pupae, dried and powdered (C).
4. Ox-liver, dried and powdered (L).
5. Excrements of rats, dried and powdered (M).
6. Bran of rice (N).
7. Ox-blood, dried and powdered (bl).
8. Haemoglobin (H).
9. Dust under the Japanese "Tatami" or dust on the floor (D).
10. Lactose, vitamins A, B, C, D and liver-oil (powdered) (K).

Results and Considerations

In order to determine whether dietal reaction would produce a differential effect upon the growth of flea larvae, experiments were conducted in which 721 0 to 24 hours old larvae were reared to maturity on diets of different types. Larvae were supplied with abundance of diets for food. The results of all experiments are summarised in tables 1 to 3.

Table 1. Effect of breeding flea larvae on various diets at room temperature and humidity. Cases fed on single diets.

Diets	Newly hatched larvae used	Larvae which reached adult stage	Larvae which reached adult stage (%)
A	30	11	36.7
B	30	0	0.0
bl	14	4	28.6
C	20	5	25.0
D	25	23	92.0
H	20	0	0.0
K	20	0	0.0
L	20	0	0.0
M	20	19	95.0

Table 2. Effect of breeding flea larvae on various diets at room temperature and humidity. Cases fed on two mixed diets.

Diets	Newly hatched larvae used	Larvae which reached adult stage	Larvae which reached adult stage (%)
A +B	30	11	36.7
A +bl	32	31	96.9
A +C	30	14	46.7

A +K	20	6	30.0
A +L	20	0	0.0
A +M	20	15	75.0
A +N	20	5	25.0
B +bl	30	18	60.0
B +C	30	11	36.7
B +L	30	0	0.0
bl +C	20	7	28.6
bl +K	20	1	5.0
bl +L	20	1	5.0
bl +M	20	0	0.0
bl +N	20	0	0.0
C +K	20	3	15.0
C +M	20	18	90.0
C +N	20	0	0.0
K +M	20	0	0.0
K +N	20	0	0.0
L +M	20	15	75.0
L +N	20	0	0.0
M+N	20	14	70.0

Table 3. Effect of breeding flea larvae on various diets at room temperature and humidity.

Diets	Duration of larval and pupal periods taken together in days			Mean relative humidity	Mean temperature (°C)
	Minimum	Maximum	Average		
A	33	81	43.3	82.7	26.0
A +B	38	126	93.2	82.8	22.6
A +C	33	48	36.0	82.5	26.5
A +bl	20	78	30.9	81.3	26.1
B +bl	23	125	31.2	81.0	26.1
B +C	34	93	44.2	82.7	25.7
D	22	40	27.3	81.2	26.9
A +K	46	91	75.8	81.8	17.1
A +M	39	88	51.2	82.3	18.5
A +N	62	98	74.0	80.9	16.8
bl	63	67	64.0	82.0	19.1
bl +C	49	99	73.5	81.9	17.1
bl +K			82.0	80.8	17.6
bl +L			49.0	83.6	20.4
C	88	94	89.2	79.8	15.5
C +K	50	51	50.3	83.5	15.5
C +M	39	50	41.8	83.8	19.6
L +M	40	65	46.1	83.5	19.3
M	40	66	45.6	83.7	19.3
M+N	24	77	49.1	81.1	17.0

Table 4. Effect of breeding flea larvae on various foods at 80 and 90 (*) percentages of relative humidity (Temperature 21°C) (Selected from E. K. Sikes, 1931).

Diets	Newly hatched larvae used	Larvae which reached adult stage	Larvae which reached adult stage (%)
* Rabbit blood	27	15	55.6
Rabbit blood and sand 1	25	17	68.0
Rabbit blood on cloth 1	25	15	60.0
* Rat blood and sand	16	9	56.3
* Human blood on cloth	25	13	52.0
Flea faeces and sand	25	15	60.0
Flea faeces on cloth 1	25	18	72.0

The present experiments indicate clearly that there are great differences in the percentage of flea larvae which reached adult stage among nine kinds of a single diet and twenty-three kinds of mixed diets. It is of interest to contrast the present results with those of similar experiments made by Sikes in 1931 (see table 4). The relatively better development of larvae and pupae, as shown by the percentage reaching the adult stage, on diets of dust under the Japanese "Tatami", or dust on the floor, or excrements of rats (dried and powdered) shows that such single diets are clearly sufficient and satisfactory for this flea. On the other hand, the relatively poor development or high mortality of larvae and pupae fed on other single diets, as well as on a number of mixed ones, indicate that even the ox-liver and blood, hitherto known as the best diets, are not as good for the post-embryonic growth of flea larvae as the single diets mentioned above. The duration of larval and pupal periods taken together, from the time of hatching from the egg to the emergence of the adult, seems to vary greatly with the kind of diet. The duration varies greatly both in the minimum and maximum number of days taken to complete the larval and pupal stages; but only the minimum number of days is most significant in the determination of dietal reaction upon the growth of flea larvae and pupae. Therefore there is a certain parallelism between the duration of the larval and pupal periods and the percentage of larvae which reach the adult stage. The experiments show that the best results are obtained in breeding flea larvae with dust under the Japanese "Tatami" or dust on the floor and excrements of ex-

crements of rats, dried and powdered. From the present experiments I would emphasize the dust and excrements of rats are the most convenient and economic diets which may be used everywhere easily in rearing experiments.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

FURTHER DATA ON THE CROSSING OF ALBINO RING DOVES WITH WHITE ONES*

MASAHARU TANGE

INTRODUCTION

In the previous paper on the studies of the sex-linked inheritance of plumage color in the ring dove, the writer (Tange, 1934) reported the results of crossing albino ring doves (which had mutated to blond ones in his aviary and were named by him "Albino I") with white ones. This cross breeding produced colored or blond individuals in F_1; that is, blond females and blond males in equal number from the crossing of a white female with an albino male, and white females and blond males in equal number from the crossing of an albino female with a white male. The results from these studies in addition to those obtained by crossing blond ring doves with white ones, so far as the plumage color was concerned, may be designated by the following genetic formulae:

Blond ring doves: ♀ = $CCZ^{I}W$, ♂ = $CCZ^{I}Z^{I}$;
White ring doves: ♀ = $CCZ^{i}W$, ♂ = $CCZ^{i}Z^{i}$;
Albino ring doves: ♀ = $ccZ^{I}W$, ♂ = $ccZ^{I}Z^{I}$.

He also considered another kind of albino ring doves, which he named "Albino II", of the genetic formulae, ♀ = $ccZ^{i}W$ and ♂ = $ccZ^{i}Z^{i}$, that was expected to be bred. The data presented

* The work was aided by the expenditure for scientific research from the Department of Education to the Laboratory for the Studies on the Genetics in Domestic Animals. Part of the experimental results was reported before the Annual Grand Meeting of the Scientific Agricultural Society held in Tokyo, 1941.

here are those of F_2's obtained by crossing the albino ring doves ("Albino I") with white ones, as well as those obtained by crossing individuals which were mainly raised out of the offspring of albino ring doves crossed with white ones.

Material and Methods

The birds used here are the white and albino ring doves above-named and the cross-bred ones obtained by crossing albino ring doves with white ones. All of them were reared in the writer's aviary and the pedigrees are known in full.

The methods of keeping birds, rearing squabs, and discriminating individuals are the same as described in the previous paper published in 1934.

Experimental Results

Some of the data concerning F_1 were presented in the previous paper: when an albino male was crossed with a white female the sons and daughters were all blond, while in the reciprocal cross, *i.e.*, a white male crossed with an albino female produced blond sons and white daughters. Further data concerning F_1, which have been obtained by continued crossing experiments show the same results, and are presented here in addition to the data concerning F_2 and others.

(A) White ♀ × Albino ♂

(a) F_1

Table 1.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ CC 95 (W)* / ♂ DD 62 (A)	7	8	0	0	0	0	15
♀ CC 95 (W)** / ♂ EE114 (A)	23	28	0	0	0	0	51

* The same pair and number of offspring as described in the previous paper (Tange, 1934).

**The same pair as described in the previous paper, but the number of offspring increased.

W=white, A=albino, H.W=hybrid white, H.B=hybrid blond.

♀ BB 5 (W) / ♂ EE 118 (A)	20	19	0	0	0	0	39
♀ CC 185 (H.W)* / ♂ EE 32 (A)	1	3	0	0	0	0	4
♀ BB 65 (H.W) / ♂ EE 32 (A)	9	14	0	0	0	0	23
♀ CC 56 (H.W) / ♂ EE 32 (A)	7	5	0	0	0	0	12
♀ CC 56 (H.W) / ♂ GG 7 (A)	48	44	0	0	0	0	92
Totals	115	121	0	0	0	0	236
Expected	118.0	118.0	0	0	0	0	236
Standard error	±7.68	±7.68					

(b) F_2

Table 2.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ FF 263 (H.B) / ♂ FF 227 (H.B)	1	5	0	0	1	0	7
♀ FF 263 (H.B) / ♂ GG 64 (H.B)	0	1	0	0	0	1	2
♀ FF 263 (H.B) / ♂ HH 163 (H.B)	11	35	23	0	16	17	102
♀ GG 134 (H.B) / ♂ GG 133 (H.B)	15	27	14	0	5	10	71
♀ GG 134 (H.B) / ♂ HH 116 (H.B)	12	9	6	0	4	8	39
♀ EE 88 (H.B) / ♂ FF 192 (H.B)	5	2	2	0	0	1	10
♀ GG 61 (H.B) / ♂ GG 62 (H.B)	20	40	25	0	14	18	117
♀ EE 187 (H.B) / ♂ EE 186 (H.B)	4	6	0	0	4	2	16
♀ EE 183 (H.B) / ♂ EE 182 (H.B)	14	21	9	0	11	15	70
♀ NN 93 (H.B) / ♂ EE 182 (H.B)	3	3	0	0	0	0	6
♀ EE 248 (H.B) / ♂ FF 14 (H.B)	3	5	4	0	6	4	22
♀ EE 248 (H.B) / ♂ GG 141 (H.B)	16	26	11	0	10	8	71
♀ MM 83 (H.B) / ♂ EE 182 (H.B)	7	12	9	0	1	1	30
♀ EE 249 (H.B) / ♂ GG 8 (H.B)	18	39	15	0	9	17	98
Totals	129	231	118	0	81	102	661
Expected	$123\frac{15}{16}$	$247\frac{1}{16}$	$123\frac{15}{16}$	0	$82\frac{10}{16}$	$82\frac{10}{16}$	661
Standard error	±10.03	±12.45	±10.03		±8.50	±8.50	

(B) Albino ♀ × White ♂

(a) F_1

Table 3.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ CC 215 (A)* / ♂ CC 96 (W)	0	3	3	0	0	0	6
♀ CC 215 (A)** / ♂ BB 11 (W)	0	29	26	0	0	0	55
♀ CC 214 (A)** / ♂ BB 54 (W)	0	25	22	0	0	0	47
♀ II 31 (A) / ♂ BB 54 (W)	0	2	2	0	0	0	4
Totals	0	59	53	0	0	0	112
Expected	0	56.0	56.0	0	0	0	112
Standard error		±5.29	±5.29				

(b) F_2.

Table 4.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ FF 269 (H.W) / ♂ FF 67 (H.B)	14	21	23	21	9	12	100
♀ FF 148 (H.W) / ♂ FF 147 (H.B)	33	21	30	21	13	27	145
♂ FF 68 (H.W) / ♀ EE 139 (H.B)	13	14	15	20	8	6	76
♀ HH 22 (H.W) / ♂ EE 139 (H.B)	13	12	10	11	8	12	66
♀ FF 1 (H.W) / ♂ EE 57 (H.B)	22	11	11	17	19	16	96
♀ FF 66 (H.W) / ♂ EE 194 (H.B)	8	5	6	8	11	8	46
♀ FF 66 (H.W) / ♂ HH 100 (H.B)	19	8	17	12	11	7	74
♀ HH 161 (H.W) / ♂ FF 195 (H.B)	14	18	17	17	16	8	90
♀ HH 142 (H.W) / ♂ FF 195 (H.B)	6	6	5	3	3	2	25
Totals	142	116	134	130	98	98	718
Expected	$134\frac{10}{16}$	$134\frac{10}{16}$	$134\frac{10}{16}$	$134\frac{10}{16}$	$89\frac{12}{16}$	$89\frac{12}{16}$	718
Standard error	±10.46	±10.46	±10.46	±10.46	±8.86	±8.86	

* The same pair and number of offspring as described in the previous paper.

**The same pair as described in the previous paper, but the number of offspring increased.

(C) Miscellaneous Crossing

(a) F_1 white ♀ of Section B.a. × "Albino I" ♂.

Table 5.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ FF 272 (H.W) / ♂ FF 197 (A)	4	5	0	0	10	5	24
♀ FF 191 (H.W) / ♂ EE 230 (A)	30	31	0	0	30	24	115
Totals	34	36	0	0	40	29	139
Expected	34¾	34¾	0	0	34¾	34¾	139
Standard error	±5.11	±5.11				±5.11	±5.11

(b) "Albino I" ♀ × F_1 blond ♂ of Section B.a.

Table 6.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ EE 47 (A) / ♂ EE 56 (H.B)	5	17	6	0	12	10	50
Expected	6⅜	12⅛	6⅜	0	12⅛	12⅛	50
Standard error	±2.34	±3.06	±2.34		±3.06	±3.06	

(c) F_2 albino ♀ × White ♂.

(i) F_2 albino ♀ of Section A.b. × White ♂.

Table 7.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ HH 36 (A) / ♂ CC 184 (W)	0	2	2	0	0	0	4
♀ HH 36 (A) / ♂ BB 54 (W)	0	2	2	0	0	0	4
♀ GG 4 (A) / ♂ DD 198 (W)	0	51	57	0	0	0	108
Totals	0	55	61	0	0	0	116
Expected	0	58.0	58.0	0	0	0	116
Standard error		±5.39	±5.39				

(ii) F_2 albino ♀ of Section B.b. × White ♂.

Table 8.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♂ GG 37 (A) / ♂ DD 198 (W)	0	0	4	2	0	0	6
♀ HH 37 (A) / ♂ CC 184 (W)	0	0	5	7	0	0	12
♀ HH 72 (A) / ♂ CC 184 (W)	0	0	1	1	0	0	2
Totals	0	0	10	10	0	0	20
Expected	0	0	10.0	10.0	0	0	20

(d) White ♀ × F_2 albino ♂ of Section B.b.

Table 9.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ BB 52 (W) / ♂ GG 22 (A)	0	0	6	6	0	0	12
Expected	0	0	6.0	6.0	0	0	12

(e) "Albino II" ♀ × White ♂.

Table 10.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ KK 22 (A) / ♂ BB 54 (W)	0	0	8	8	0	0	16
Expected	0	0	8.0	8.0	0	0	16

(f) "Albino II" ♀ × Parent of albino mutants.

Table 11.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ JJ 5 (A) / ♂ BB 61 (B)	8	11	0	0	13	8	40

♀ JJ 92 (A) / ♂ BB 10 (B)	11	18	0	0	9	15	53
Totals	19	29	0	0	22	23	93
Expected	23½	23½	0	0	23½	23½	93
Standard error	±4.18	±4.18			±4.18	±4.18	

(g) F_1 white ♀ of Section B.a. × Albino ♂ of Section C.b

Table 12.

Pair	Blond		White		Albino		Totals
	♀	♂	♀	♂	♀	♂	
♀ FF 271 (W) / ♂ FF 193 (A)	8	5	9	6	13	20	61
Expected	7⅝	7⅝	7⅝	7⅝	15⅜	15⅜	61
Standard error	±2.58	±2.58	±2.58	±2.58	±3.38	±3.38	

(D) Pure Breeding of "Albino II"

Table 13.

Pair	Albino		Totals
	♀	♂	
♀ HH 72 (A) / ♂ GG 22 (A)	41	49	90
♀ II 19 (A) / ♂ KK 4 (A)	13	17	30
♀ LL 32 (A) / ♂ MM 43 (A)	6	5	12
♀ LL 17 (A) / ♂ LL 45 (A)	21	26	47
♀ LL 2 (A) / ♂ LL 1 (A)	30	28	58
♀ KK 1 (A) / ♂ KK 28 (A)	26	25	51
Totals	137	150	287
Expected	143.5	143.5	287
Standard error	±8.47	±8.47	

(E) To Distinguish "Albino II" from "Albino I"

(i) To distinguish "Albino II" ♀ from "Albino I" ♀.

Table 14.

Pair	Blond		White		Totals
	♀	♂	♀	♂	
♀ HH 72 (A) / ♂ CC 133 (W)	0	0	1	1	2
♀ II 19 (A) / ♂ CC 133 (W)	0	0	1	1	2

Each of the birds ♀ HH 72 (A) and ♀ II 19(A) produced a white male offspring from the crossing with white male, and this enables us to regard the birds as "Albino II".

(ii) To distinguish "Albino II" ♂ from the Albino ♂ which is heterozygous with respect to the gene *I*.

Table 15.

Pair	Blond		White		Totals
	♀	♂	♀	♂	
♀ BB 52 (W) / ♂ KK 4 (A)	0	0	5	5	10
Expected	0	0	5.0	5.0	10
Standard error			±1.58	±1.58	

The results obtained from the crossing metioned above are enough to discriminate the bird ♂ KK 4 (A) to be the "Albino II" ♂, for the reason described in Section 3. a. ii. under Consideration (p. 140).

Consideration

(1) F_1 and F_2. The experimental results of crossing albino ring doves ("Albino I") with white ones were described in the previous paper, and assumptions were made: the blond color of plumage in the ring dove was due to two factors which were named by the writer "C" and "I", *C* being an autosomal color factor and responsible for developing the plumage color and eye

color characteristic to the white ring dove, *I* being a sex-linked intensity factor unable to exhibit any coloration by itself and causing the bird to remain an albino. The genetic formulae for the white and albino ring doves were designated as follows:

White ring doves: ♀ = $CCZ^{I}W$, ♂ = $CCZ^{I}Z^{I}$;
Albino ring doves: ♀ = $ccZ^{I}W$, ♂ = $ccZ^{I}Z^{I}$.

The results shown in Tables 1, 2, 3 and 4 may be interpreted by the following scheme of inheritance:

(A)

White ♀ × Albino ♂

P_1	$CCZ^{I}W$ white ♀	×	$ccZ^{I}Z^{I}$ albino ♂
P_1 gametes	(CW), (CZ^{I})		(cZ^{I})
F_1	$CcZ^{I}W$ blond ♀		$CcZ^{I}Z^{I}$ blond ♂
	1	:	1
F_1 gametes	(CW), (cW), (CZ^{I}), (cZ^{I})		(CZ^{I}), (CZ^{I}), (cZ^{I}), (cZ^{I})

F_2	$CCZ^{I}W$ blond ♀	$CCZ^{I}W$ white ♀	$CcZ^{I}W$ blond ♀	$CcZ^{I}W$ white ♀
	$CcZ^{I}W$ blond ♀	$CcZ^{I}W$ white ♀	$ccZ^{I}W$ albino ♀	$ccZ^{I}W$ albino ♀
	$CCZ^{I}Z^{I}$ blond ♂	$CCZ^{I}Z^{I}$ blond ♂	$CcZ^{I}Z^{I}$ blond ♂	$CcZ^{I}Z^{I}$ blond ♂
	$CcZ^{I}Z^{I}$ blond ♂	$CcZ^{I}Z^{I}$ blond ♂	$ccZ^{I}Z^{I}$ albino ♂	$ccZ^{I}Z^{I}$ albino ♂

blond ♀ : blond ♂ : white ♀ : white ♂ : albino ♀ : albino ♂
3 : 6 : 3 : 0 : 2 : 2

(B)

	Albino ♀	×	White ♂
P_1	$ccZ^{I}W$ albino ♀	×	$CCZ^{I}Z^{I}$ white ♂
P_1 gametes	(cW), (cZ^{I})		(CZ^{I})
F_1	$CcZ^{I}W$ white ♀		$CcZ^{I}Z^{I}$ blond ♂
	1	:	1

F_1 gametes	(CW), (cW), (CZ^i), (cZ^i)		(CZ^I), (CZ^i), (cZ^I), (cZ^i)	
F_2	CCZ^{I}W blond ♀	CCZ^{i}W white ♀	CcZ^{I}W blond ♀	CcZ^{i}W white ♀
	CcZ^{I}W blond ♀	CcZ^{i}W white ♀	ccZ^{I}W albino ♀	ccZ^{i}W albino ♀
	CCZ^{I}Z^i blond ♂	CCZ^{i}Z^i white ♂	CcZ^{I}Z^i blond ♂	CCZ^{i}Z^i white ♂
	CcZ^{I}Z^i blond ♂	CcZ^{i}Z^i white ♂	ccZ^{I}Z^i albino ♂	ccZ^{i}Z^i albino ♂
	blond ♀ : blond ♂	white ♀ : white ♂	albino ♀ : albino ♂	
	3 : 3	3 : 3	2 : 2	

As is shown in Tables 1, 2, 3 and 4, the actual numbers of offspring, *i.e.*, F_1 and F_2 *ex* White ♀ × Albino ♂ as well as those *ex* Albino ♀ × White ♂, are not far from the theoretical ones calculated upon the above scheme; deviations do not exceed three times the standard errors.

(2) Various Cases of Crossing. The results described in Tables 5, 6, 7, 8, 9, 10, 11 and 12 are obtained from the miscellaneous crossing, and may be interpreted by the same scheme as described above. Thus:

(a) F_1 white ♀ of Section B. a. × "Albino I" ♂. (Table 5).

The F_1 white ♀ is heterozygous with respect to the gene *C*, and produces four kinds of gametes instead of two (Cf. P_1 of Section A). Consequently there occurs both blond and albino offspring in F_1 when it is mated with "Albino I" ♂; it differs from F_1 *ex* pure White ♀ × "Albino I" ♂:

P_1	CcZ^{i}W		× ccZ^{I}Z^I	
P_1 gametes	(CW), (CZ^i), (cW), (cZ^i)		cZ^I	
F_1	CcZ^{I}W blond ♀	CcZ^{I}Z^i blond ♂	ccZ^{I}W albino ♀	ccZ^{I}Z^i albino ♂

The experimental results shown in Table 5 are in agreement with the theoretical expectation.

(b) "Albino I" ♀ × F_1 blond ♂ of Section B. a. (Table 6).

The F_1 blond ♀ is heterozygous with respect to genes *C* and

I, and generates four kinds of gametes. When it is crossed with all "Albino I" ♀ all appear blond, white and albino offspring except white ♂.

P_1	ccZ^IW	×	CcZ^IZ^i		
P_1 gametes	$(cW), (cZ^I)$		$(CZ^I), (CZ^i), (cZ^I), (cZ^i)$		
F_1	CcZ^IW	CcZ^iW	ccZ^IW	ccZ^iW	
	blond ♀	white ♀	albino ♀	albino ♀	
	CcZ^IZ^I	CcZ^IZ^i	ccZ^IZ^I	ccZ^IZ^i	
	blond ♂	blond ♂	albino ♂	albino ♂	
blond ♀	blond ♂	white ♀	white ♂	albino ♀	albino ♂
1 :	2 :	1 :	0 :	2 :	2

The results shown in Table 6 agree with the theoretical expectation, although the actual numbers of progeny are not large.

(c) F_2 albino ♀ × White ♂. (Tables 7 and 8).

There are two kinds of F_2 albino ♀ *ex* both White ♀ × Albino ♂ and Albino ♀ × White ♂, and they are to be designated genetically as ccZ^IW and ccZ^iW as shown in the scheme described above. When they are crossed with White ♂, the former produces F_1's of blond ♂ and white ♀ in equal number, while the latter produces F_1's of white ♀ and white ♂ in equal number, no blond offspring at all in this case; for example, HH 36 shown in Table 7 is an F_2 albino ♀ of Section A. b. and GG 37 is an F_2 albino ♀ of Section B. b., the former is to be designated as ccZ^IW and the latter as ccZ^iW: there are F_2 albino ♀'s which are to be designated as ccZ^iW in Section A. b., and there are also F_2 albino ♀'s which are to be designated as ccZ^IW in section B.b. The scheme of inheritance is as follows:

(i) P_1	ccZ^IW	×	CCZ^iZ^i		
P_1 gametes	$(cZ^I), (cW)$		(CZ^i)		
F_1	CcZ^iZ^i		CcZ^iW		
	blond ♂		white ♀		
	1	:	1		
(ii) P_1 gametes	ccZ^iW	×	CCZ^iZ^i		
P_1 gametes	$(cW), (cZ^i)$		(CZ^i)		
F_1	CcZ^iW		CcZ^iZ^i		
	white ♀		white ♂		
	1	:	1		

All the results of crossing are in agreement with the theoretical expectation although the number of individuals is small.

The albino females lacking in *I* are the "Albino II" females named so by the writer.

(d) White ♀ × F_2 albino ♂ of Section B.b. (Table 9).

There are two kinds of F_2 albino ♂ of Section B.b., *i.e.*, ccZ^IZ^I and ccZ^IZ^i. When they are crossed with white ♀, the former produces both blond and white offspring, while the latter produces white offspring only; F_2 albino ♂ such as GG22 in Table 9 must be an individual of the genetic constitution of ccZ^iZ^i. The results of crossing white ♀ with F_2 albino ♂ of the genetic constitution of ccZ^IZ^i have not been obtained in the present crossing experiments.

(i) P_1	CCZ^iW	×	ccZ^IZ^I					
P_1 gametes	$(CW), (CZ^i)$		(cZ^I) (cZ^I)					
F_1	CcZ^IW blond ♀		CcZ^IZ^I blond ♂		CcZ^iW white ♀		CcZ^IZ^i white ♂	
	1	:	1	:	1	:	1	
(ii) P_1	CCZ^iW	×	ccZ^iZ^i					
P_1 gametes	$(CW), (CZ^i)$		(cZ^i)					
F_1	CcZ^iW white ♀		CcZ^iZ^i white ♂					
	1	:	1					

The results of crossing shown in Table 9 correspond to the scheme described in (ii) above and the bird GG22 is to be regarded as an "Albino II" ♂.

(e) "Albino II" ♀ × White ♂. (Table 10).

As the albino ♀ KK22 shown in Table 10 is the female offspring *ex* ♀ HH72 (A) in Table 8 × ♂ GG22 (A) in Table 9, the former being of the genetic constitution of ccZ^iW and the latter of ccZ^IZ^i (Cf. the preceding Section), it belongs only to "Albino II", and the crossing of the present Section is identical with that of Section c. ii.

P_1	ccZ^iW	×	CCZ^IZ^i
P_1 gametes	$(cW), (cZ^i)$		(CZ^I)

F_1	CcZ^1W		CcZ^1Z^1	
	white ♀		white ♂	
	1	:	1	

(f) "Albino II" ♀ × Parent of albino mutants. (Table 11).

As the male parent of albino mutants may be designated as CcZ^1Z^1 (Tange, 1934), the results of crossing "Albino II" ♀ with the male parent are able to be explained by the following scheme of inheritance:

P_1	ccZ^1W			×	CcZ^1Z^1		
P_1 gametes	$(cW), (cZ^1)$				$(CZ^1), (cZ^1)$		
F_1	CcZ^1W	CcZ^1Z^1			ccZ^1W	ccZ^1Z^1	
	blond ♀	blond ♂			albino ♀	albino ♂	
	1	: 1	:		1	: 1	

The results of crossing are in approximate agreement with the theoretical expectation as shown in Table 11.

(g) F_1 white ♀ of Section B.a. × Albino ♂ of Section C.b. (Table 12).

The genetic constitution of the F_1 white ♀ of Section B.a. is CcZ^1W as shown above. There are two kinds of genetic constitution of albino ♂ of Section C.b., *viz.*, ccZ^1Z^1 and ccZ^1Z^1, the former belonging to "Albino I". The male bird FF193(A) in Table 12 is considered to be designated as ccZ^1Z^1 instead of ccZ^1Z^1, because there occurred white offspring when it was crossed with female white bird, (Cf. Section C.a.). Thus:

P_1	CcZ^1W				×	ccZ^1Z^1				
P_1 gametes	$(CW), (cW), (CZ^1), (cZ^1)$					$(cZ^1), (cZ^1)$				
F_1	CcZ^1W	CcZ^1W			ccZ^1W	ccZ^1W				
	blond ♀	white ♀			albino ♀	albino ♀				
	CcZ^1Z^1	CcZ^1Z^1			ccZ^1Z^1	ccZ^1Z^1				
	blond ♂	white ♂			albino ♂	albino ♂				
	blond ♀	blond ♂	white ♀	white ♂	albino ♀	albino ♂				
	1	: 1	: 1	: 1	: 2	: 2				

The results of crossing shown in Table 12 are well explained

by the scheme mentioned above, the actual numbers secured being not far from the theoretical ones; the deviations do not exceed three times the standard errors.

(3) The "Albino II" Individuals Breed True.

(a) To distinguish genotypically the "Albino II" from the "Albino I" by the progeny test. (i) To distinguish genotypically the "Albino II" ♀ from the "Albino I" ♀. This is simple since the "Albino I" ♀ produces male offspring of blond plumage color when it is crossed with White ♂, while the "Albino II" ♀ does not produce such colored offspring when crossed with White ♂ (Cf. Sections c.i., c.ii. and e.). The female birds HH72(A) and II19(A) shown in Table 14 can be considered as individuals belonging to "Albino II", because both of them produced *male* offspring of white plumage color. (ii) To distinguish genotypically the "Albino II" ♂ (ccZ^lZ^l) from the "Albino I" ♂ (ccZ^lZ^l) as well as from the Albino ♂ which is heterozygous with respect to the gene *I*, *i.e.*, Albino ♂ designated as ccZ^lZ^i. The "Albino I" ♂ produces *blond* offspring only in F_1 when it is crossed with the white female as was shown in Table 1, while the "Albino II" ♂ produces *white* offspring only in F_1 when it is crossed with the white female as was explained in Section d.ii. Thus the "Albino II" ♂ may be distinguished from the "Albino I" ♂ without difficulty by the progeny test. We will consider next the method of distinguishing the "Albino II" ♂ from the Albino ♂ heterozygous with respect to the gene *I*. The Albino ♂ designated as ccZ^lZ^i produces both blond and white offspring in equal number as was shown in the scheme of inheritance described in Section d.i., while the "Albino II" ♂ produces white offspring only when crossed with the white female. So if the total number of offspring produced by an albino ♂ crossed with a white ♀ is at least 10 and all are white without exception, this enables us to determine the albino ♂ to be an "Albino II" ♂ and never to be an Albino ♂ of ccZ^lZ^i; thus the deviation and standard error of experiments calculated on the assumption that the ratio of the blond birds to the white ones which will be produced by crossing Albino ♂ of ccZ^lZ^i with white ♀ of CCZ^lW is 1:1, are ±5.0 and ±1.58, respectively; the deviation exceeds three times the standard error,

so the experimental results, which secure 10 birds of white plumage without exception, are not explainable by the scheme of $CCZ^{I}W \times ccZ^{I}Z^{I}$ (Cf. Sections d. i. and d. ii.).

(b) The "Albino II" individuals breed true. An F_2 albino female HH72 was mated with an F_2 albino male GG22 as shown in Table 13, both of them being the progeny *ex* Albino ♀ × White ♂ of Section B, and having been shown, as mentioned above, that they are of the genetic constitution $ccZ^{I}W$ and $ccZ^{I}Z^{I}$, respectively. They have bred 90 individuals up to the present, all albino, 41 being females and 49 males. The other five pairs shown in Table 13 are all the offspring *ex* HH72 × GG22. Two individuals ♀ II 19 as well as KK4 out of 10 have shown that they have no *I* gene in their genetic constitution as a result of crossing them with white birds as shown in Tables 14 and 15; the remaining eight individuals are sure to be of the same genetic constitution as the two. Thus a new kind of albino ring doves of the genetic constitution ♀ = $ccZ^{I}W$ and ♂ = $ccZ^{I}Z^{I}$ have been bred, and they breed true.

Summary and Conclusion

(1) Further data on the crossing of albino ring doves with white ones are presented in this paper.

(2) It is shown that the results of crossing experiments presented in this paper are all satisfactorily explained by the two-factor hypothesis brought forward by the writer (Tange, 1934). The blond color of plumage in the ring dove is developed by the combination of two factors: one being that which is responsible for the white plumage color and the dark eye color characteristic of the white ring dove, and the other being the intensity factor. The intensity factor can not develop coloration by itself, therefore it leaves the bird an albino with the snow-white plumage and the red eye. The two factors are the color factor *C* and the intensity factor *I*, the former being autosomal and the latter sex-linked as described in a paper published in 1934.

(3) Albino ring doves lacking both *C* and *I* factors and designated as ♀ = $ccZ^{I}W$ and ♂ = $ccZ^{I}Z^{I}$ have been bred by the present crossing experiments. They breed true and have established

another kind of albino ring doves which the writer named "Albino II".

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Kyushu University, July, 1948.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

THE GONIMOBLAST DEVELOPMENT IN CERAMIACEOUS ALGAE OF JAPAN. I*

SOKICHI SEGAWA

The developmental studies of the reproductive organs in the algae of the family Ceramicaceae were carried out extensively by Kylin[1], who used the materials taken mostly from the Atlantic ocean. The investigations of Japanese members of this group are of interest.

Callithamnion callophyllidicola Yamada

As to the gonimoblast development of this alga, the writer previously made some studies published in Japanese.[2] The material was collected by the writer from Susaki, Izu Province. The identification of the material used was ascertained by comparison with that of Enoshima, the type locality of this species.

The carpogonial branch arises on an intercalary vegetative cell in the upper normal branch of this weed. By this central cell two pericentral cells are laterally produced on both sides of it; later each cell in question becomes an auxiliary mother cell, one of two lateral cells of the central cell becomes a supporting cell bearing a carpogonial branch composed of four cells. Thus, the fertile central cell of the upper normal branch has two lateral cells and a four-celled carpogonial branch supported by one of the lateral cells.

* Contribution from the Fisheries Laboratory, Faculty of Agriculture, Kyūshū University, Fukuoka, & the Mitsui Institute of Marine Biology, Susaki near Shimoda, Izu.

After fertilization each auxiliary mother cell cuts off an auxiliary cell upwards. Each auxiliary cell issues a process towards the fertilized carpogonium. At the same time the fertilized carpogonium becomes inflated horizontally and cuts off a somewhat large cell by an oblique vertical wall. At the advanced stage the latter cell produces one small cell, and the fertilized carpogonium itself also cuts off a small cell. These cells newly produced from the carpogonium are the connecting cells. Therefore it is concluded that the carpogonium issues a connecting cell and a two-celled connecting filament. The auxiliary cell produced from the supporting cell fuses with a connecting cell mentioned above.

After fusion each auxiliary cell produces an initial cell of gonimoblast.

Antithamnion Plumula (Ellis) Thuret

The gonimoblast development of this species has been studied by Phillips (1897)[3] and more fully by Kylin (1923)[1] in European materials. The material used by the present writer was collected from Onomichi, Bingo Province.

The carpogonial branch arises on the undermost cell of the pinnula of a few cells near the apex. The supporting cell corresponds to a pericentral cell of the central axis. The central cell bearing the pinnula and the supporting cell do not differ much from other vegetative cells. The carpogonial branch is four-celled and stands upwards in a curve on one side of the supporting cell.

After fertilization an auxiliary cell is cut off upwards from the supporting cell, and the auxiliary cell is situated near the fertilized carpogonium. The carpogonium cuts off a small cell upwards by a horizontal wall and then another small cell laterally by an oblique vertical wall. The former lies near the base of the trichogyne, and seems to correspond with "a small mass of granular matter" described by Phillips. The latter small cell fuses with the auxiliary cell.

The gonimoblast initial is produced by the auxiliary cell upwards. The gonimoblast consists of 2 or 3 gonimolobes produced from the initial. The gonimoblast is surrounded by involucres produced from central cells near to it.

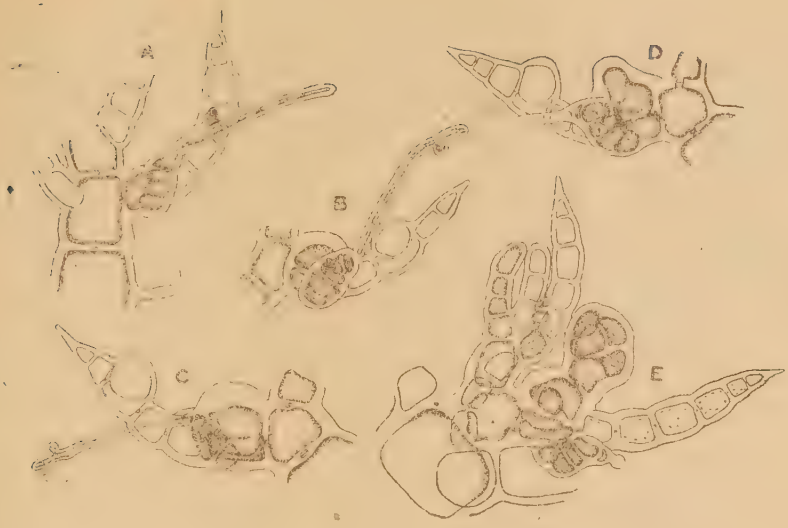

Fig. 1. *Antithamnion Plumula.*
a. procarp.
b-d. procarp after fertilization.
e. development of gonimoblast.
× 300

Spermothamnion Tamamiru Segawa

The material of this study was collected from Susaki, Izu Province. The species is rather common in this locality and found on *Codium mamillare* as an endophytic alga.

The fertile part of the female individual is on the top of the erect filament or its branchlet, and consists of three differentiated cells of small size. The middle central cell of three becomes fertile, and cuts off three pericentral cells, the second and third developing one on either side of the first. Then the second pericentral cell cuts off a small cell, which is sterile. After this the former becomes the supporting cell of the four-celled carpogonial branch. The carpogonial branch grows exactly opposite to the first pericentral cell.

After fertilization the second and third cells become the auxiliary mother cell, and each cuts off an auxiliary cell upwards. On the other hand the fertilized carpogonium cuts off two connecting cells of small size on both sides of it. Then these cells fuse each other with two auxiliary cells mentioned above.

Each auxiliary cell produces an initial cell of gonimoblast. From the outermost cells of the gonimoblast filaments the carpospores are produced. There are no involucres at all around the gonimoblast.

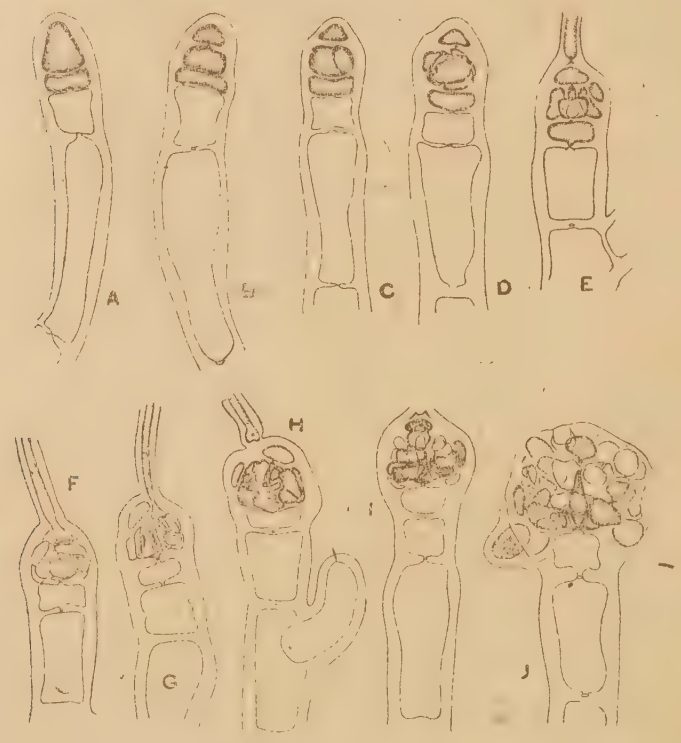

Fig. 2. *Spermothamnion Tamamiru*.
a–g. development of procarp.
h. procarp after fertilization.
i–j. development of gonimoblast.
× 250

Here the writer wishes to express his hearty thanks to Prof. Dr. Y. Yamada for his kind direction. Thanks are also due to Prof. Dr. K. Uchida and Prof. Dr. I. Amemiya for their kind help.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

ÜBER DIE EINWIRKUNG VON HYPOHALOGENIT AUF METHYLFURFURAL*

Kazuyuki Maekawa

Wenn Methylfurfural in einer 12%igen Salzsaurelösung in kochendem Wasserbad erhitzt wird, so vermehrt sich die Menge der Substanz, die von der Natrium-hypojoditlösung reagiert wird, während sich die Menge des mit Phloroglucin niederfällbaren Methylfurfurals vermindert. Diese erstmals entdeckte Tatsache führte Verfasser vorläufig auf die Ringöffnung des Furankerns zurück, und kam dadurch zur fraktionierten jodometrischen Bestimmung von Furfural und Methylfurfural.[1] So wird die leichtere Zersetzbarkeit von Methylfurfural als Furfural wohl dem Einflusse der ω-ständigen Methylgruppe zukommen. Um weiteres darüber zu klären, untersuchte Verfasser hinsichtlich der Einwirkung von Hypohalogenit auf Methyl-furfural und -brenzschleimsäure.

Zur Entstehung des Haloforms aus Methylketon ist Enolisation von Keton in erster Linie unentbehrlich, und auf der folgenden Stufe setzt sich ihr strenges positives Wasserstoffatom mit Halogenatom um.[2][3][4][5][6] Wenn man dieses nun zu Fällen von Methylfurfural und Methylbrenzschleimsäure anführt, so bedarf es der Enolisation von der ω-ständigen Methylgruppe für die Entstehung des Haloforms von beiden mit Hypo-bromit und -jodit. Für diese Enolisierbarkeit muss sich der Furankern entweder hydrolytisch[7][8] oder durch einen peroxydischen Zwischenzustand[9][10] oder in anderer Weise[11][12][13] sprengen. Das Wasserstoffatom der ω-ständigen Methylgruppe kann sich nämlich nicht an der Enolisation beteiligen, falls Sauerstoff in dem gebundenen,

* Vorgetragen vor dem Bezirksverein West-Japan von der Agr. Chem. Soc. Japan mit Prof. M. Hamada gemeinsam am 28. Feb. 1942; J. Agr. Chem. Soc. Japan, 18 (1942), 24 B. Untersuchungen über Furfural, IV. Mitteilung.

bruckenartigen Zustand gewesen ist. Wenn Methylfurfural sich in diesem Falle nach dem von Pummerer[6] vorgeschlagenen Zersetungsprozess verhielte, so würde man Bernsteinsäure gewinnen. In Wirklichkeit isolierte Verfasser aber aus dem Reaktionsgemisch von Hypohalit und Methylfurfural sowie Hypohalit und Methylbrenzschleimsäure Maleinsäure. Also wird es sich wohl in diesem Falle nicht hydrolytisch, sondern in anderer Weise, insbesondere unmittelbar Diketon bildend verhalten haben.

Als zweites Produkt von Methylfurfural und Methylbrenzschleimsäure wurde Bromoform sichergestellt und bestimmt. Die dabei gefundenen Werte ergeben, dass, falls 1 Mol. Methylfurfural und Methylbrenzschleimsäure 1 Mol. Bromoform liefert, 85% des Ausgangsmaterials umgewandelt worden sind. (Während Jodoform mit Hypojodit nur 6% ergeben hat.) Wenn die Reaktion von Methylfurfural und Hypobromit im Kaltgemische durchgeführt wird, so findet nur Oxydation von Methylfurfural zur Methylbrenzschleimsäure statt, ohne weitere Reaktion.

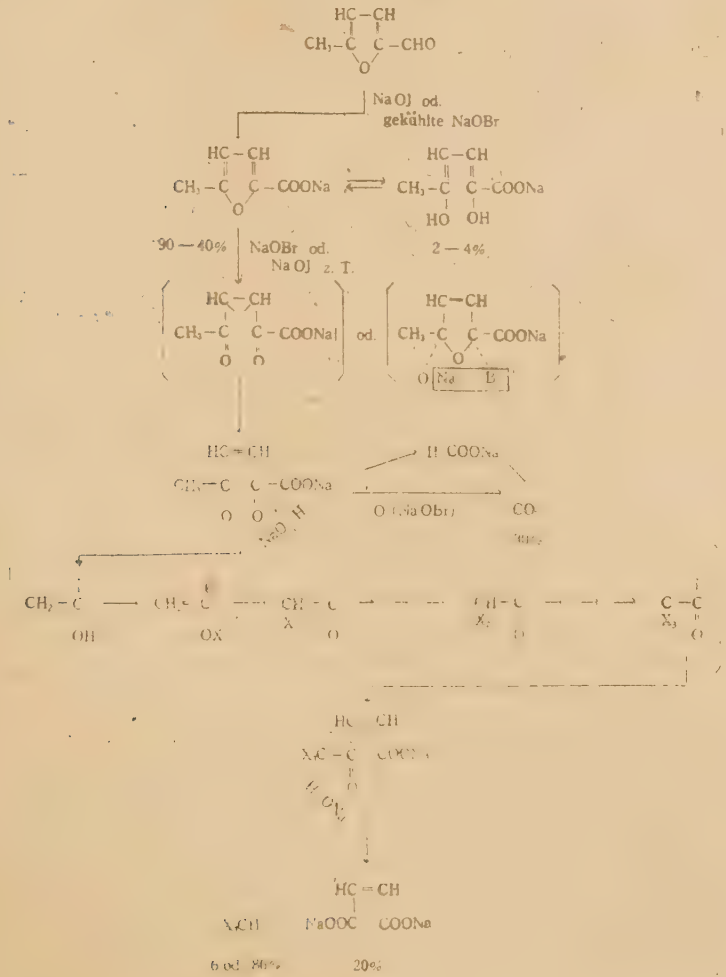
Das im Reaktionsverlauf entwickelte Kohlensäuregas wurde auch sichergestellt und quantitativ ermittelt aus folgenden Gründen, 1. als mittelbarer Hinweiss hinsichtlich der Entstehung von 1,4 Diketon, 2. um oxydative Zersetzung von α-Ketonsäure aufzuklären, und 3. um die Tatsache ans Licht zu fördern, dass die α-ständige Methylgruppe der Decarboxylierungsfähigkeit der Karbonsäure teilhaft ist. Die dabei gefundenen Werte ergeben, dass, falls 1 Mol. Probe 1 Mol. CO_2 liefert, sich nur 30% von der theoretischen Ausbeute entwickeln.

Das verbrauchte Bromatom war auch hinsichtlich des Methylfurfurals und der Methylbrenzschleimsäure bzw. etwa 7.5 Atome und 5.5 Atome. Dies zeigt, dass bei jenem sowohl Sprengung des Furankerns und Entstehung von Bromoform als auch Oxydation von Aldehyd zur Carboxylgruppe stattgefunden haben.

Zuletzt in Bezug auf frühere Mitteilung[1] wurde die jodometrische Titration über die Methylbrenzschleimsäure sofort und nach einstündiger Erhitzung mit oder ohne verd. Salzsäure ausgeführt. Die dabei gefundenen Ergebnisse zeigen, dass Methylbrenzschleimsäure in wässriger Lösung eine sehr kleine Menge von Jod verbraucht hat, und also 2–4% davon in der mit Hypojodit oxydierbaren Form immer existieren. Wenn sie in wässriger Lösung erhitzt oder stehengelassen wird, so vermehrt sich freilich

der Jodverbrauch. Erhitzt man auch Methylbrenzschleimsäure in 12%iger Salzsäurelösung, vermehrt sich der Jodverbrauch soweit und färbt sich so gelb bis orange[11] ($\lambda = 460$ mμ), dass man damit beide, Brenzschleimsäure und Methylbrenzschleimsäure, unterscheiden kann, weil die erstere nicht verbraucht wird und sich nicht färbt.

Demnach folgert Verfasser hinsichtlich der Einwirkung von Hypohalit auf Methylfurfural und Methylbrenzschleimsäure folgendes:



Me-furfûral wird nämlich durch Hypojodit und abgekühltes Hypobromit zum Me-brenzschleimsäuressalz oxydiert, in welchem 2-4% in verdünnter wässriger Lösung (wie 10 milli Mol.) als Form derjenigen vorhanden ist, wo mit Alkali und Jod Jodoform entstehen kann, und die Deformierung nach dieser Form beim Erhitzen mit 12%iger Salzsäure gefördert wird. Hypobromit wirkt weiter auf Me-brenzschleimsäure ein; hierauf öffnet sich der Furankern zuerst, und eine Seite der Spaltstücke, nämlich Methylketon wird sofort[6][15] durch übriges Alkali enolisiert, schliesslich entsteht $CHBr_3$; und die andere Seite entlässt unmittelbar oder nach dem Verlauf der Ameisensäure CO_2-Gas. (Gleichfalls entsteht CO_2-Gas aus Ameisensäure[16]).

Verfasser folgert, dass auch bei Hypojodit der Verlauf zum Teil in gleicher Weise ist.

Die obengenannten Überlegungen könnten weiter für die Aufklärung gleicher Erscheinungen geltend gemacht werden, dass Pyronring,[17] Laktonring[15] usw. durch Hypohalit die Ringe öffnen und dabei Haloform entsteht.

Beschreibung der Versuche

I Einwirkung von Na-hypobromit auf Me-furfural und Me-brenzschleimsäure

(a) Zahl der verbrauchten Bromatome pro Mol. Subst.

Je 20 ccm Bromlösung (enthielt 30 g KBr und 8 g Br_2 im L. und wurde hinsichtlich ihrer gewissen Menge vorläufig KJ versetzt und mit 0.1 n-Thiosulfat titriert) und 0.5 n-NaOH in veränderlicher Menge wurden in die Erlenmeyersche Flasche, die mit einem Stopfen versehen ist, gegossen, und die rote Farbe des Broms veränderte sich zu hellgelb. Dann wurden je 25 ccm 1 milli Mol. Lösung von Me-furfural bzw. Me-brenzschleimsäuresnatrium hinzugefügt und gut geschüttelt. Nach einstündigem Stehenlassen wird ein wenig Kaliumjodid zugesetzt, mit verd. Schwefelsäure angesäuert geschüttelt, und titriert dadurch frei werdendes Jod mit 0.1 n-Thiosulfat. Die dabei gefundenen Ergebnisse sind folgende;

Tabelle I. Einwirkung von NaBrO auf Me-furfural und Me-brenzschleimsäure.

0.5 n-NaOH hinzugefügt in ccm	Methylfurfural Bromverbrauch in ccm	mg	Atome	Methylbrenzschleimsäure Bromverbrauch in ccm	mg	Atome
4.00 (äquiv. zu Br)	18.63	149.06	7.45	13.35	106.82	5.34
4.25	18.63	149.06	7.45	13.50	108.03	5.40
4.50	18.50	148.00	7.40	13.27	106.17	5.31
5.00	18.35	146.80	7.34	13.63	109.04	5.45
5.50	16.81	134.45	6.72	13.15	105.20	5.26
6.00	12.38	99.05	4.95	13.39	107.18	5.38
7.50	6.57	52.58	2.63	13.59	108.73	5.44
10.00	5.46	43.67	2.18	18.23	145.82	7.29

(10 ccm Br-lsg.=80 mg Br_2)

Nämlich, durch Zusatz von theoretisch geforderter Alkalimenge verbraucht Me-furfural etwa 7.5 Atome Brom und Me-brenzschleimsäuresnatrium nur etwa 5.5 Atome Brom. Aber durch weiteren Alkalizusatz ergibt sich ein umgekehrtes Verhältnis.

(b) Bestimmungen von entstandenem Bromoform.

3.2 g Brom und 100 ccm 0.5 n-NaOH werden in birnförmigen Kolben mit einem unter ihrem Boden gestellten, in Grade eingeteilten Rohr zugesetzt, dann wird 1.1 g Me-furfural (in 100 ccm wässriger lösung) hinzugefügt und geschüttelt. Die klare Lösung wird bald trüb und scheidet zuletzt eine hellgelbe, schwere Lösung, nämlich $CHBr_3$, ab, das sich in dem unter dem Boden gestellten Rohr sammelt. Ausbeute etwa 0.75 ccm (86% der für 1 Mol. $CHBr_3$ aus 1 Mol. Me-furfural berechneten Menge).

K_{760} 146.0° (korr.) n_D^{18} 1.596 D_D^{20} 2.8875

21.062,25.102 mg Sbst.: 46,871, 55.824 mg AgBr

$CHBr_3$ Ber. Br. 94.86%

Gef. „ 94.70, 94.64%

(Anhang; Bestim. von der durch Elektolyse entstandenem Jodoform)

1.1 g Me-furfural löst man in 100 ccm Wasser, hierin wird 10 g KJ und 5 g Na_2CO_3 zugesetzt, und elektrolysiert[18];

Pt Anod, Pb-Kathod in der Tonzelle. Stromdichte 1 Ampere/Dm^2 und CO_2-Gas einleitend. Elektrolysat wird in schwachem Alkali mit Äther ausgezogen und CHJ_3 nach Slottaschem Verfahren[25] bestimmt.

Zwischen der Entstehung von CHJ_3 und Alkalinität des Reaktionsgemisches bestanden keine Beziehungen. Diese Ergebnisse zusammengefasst sind folgende.

(c) Zu 5.5 g Me-furfural (6.3 g Me-brenzschleimsäure) in 500 ccm Wasser gibt man bei Zimmertemperatur eine Lösung von 16 g Brom und 9.6 g Ätznatron in 200 ccm Wasser. Nach eintägigem Stehen bei Zimmertemperatur wird die Lösung vom dem sich am Boden sammelnden Bromoform abgetrennt, einige Stücke KJ zugesetzt, mit verd. H_2SO_4 angesäuert, (wenn dabei Jod frei geworden ist, wird es mit Natriumsulfit reduziert) mit Natriumsulfat gesättigt und etwa 20 mal mit Äther ausgezogen. Der im Vacuum getrocknete Ätherrückstand erstarrt bald zu einem harten Kristall, der aus Wasser umkristallisiert wird, Ausbeute ca. 1 g. F. 129.5°, mit Maleinsäure keine Schmelzpunkterniederung zeigt.

47.2 , 86.5 mg	Subst. verb. gegen Phenolpthalein
8.05 , 14.77 ccm	n/10 Natronlauge, während für Maleinsäure
8.14 , 14.91 ccm	berechnet wird.

Nachdem das aus Me-furfural und Bromlauge bestehende Reaktionsgemisch einige Minuten lang den Sonnenstrahlen ausgesetzt worden war, wurden Bariumchlorid, Ammoniak und Alkohol in einer 3 mal so grossen Menge zugesetzt.[12][19] Der dabei ausgefällte Niederschlag von Bariumsalz wurde filtriert, mit verd. Ammoniak gewaschen und wieder mit 5%iger Salpetersäure aufgelöst. Die Säure wurde aus der von Halogen befreiten Flüssigkeit von 5%iger Salpetersäure mit Merkuronitrit in 5%iger Salpetersäure als Merkurofumarat gefällt. Nach Stehenlassen im Eisschrank wurde der Niederschlag durch Glasfiltertiegel

Tabelle II. Jodoform aus Methylfurfural.

Alkalinität der Reaktionsgemische	mg CHJ_3	Mol. CHJ_3
0.06 n (0.11 g Probe)	21.8	0.06
0.28 〃 (〃)	22.0	〃
0.53 〃 (〃)	21.9	〃
1.80 〃 (〃)	22.2	〃
Bei Elektrolyse (Probe 1.1g)	224.7	〃
	221.1	〃

Alle zeigten nur 6% der für 1 Mol. CHJ_3 aus 1 Mol. Me-furfural berechneten Menge. Wird Me-furfural in Salzsäurelösung erhitzt, so dabei wird die Zersetzung nach einer Richtung veranlassen, die Entstehung von CHJ_3 mit Alkali und Jod vermehrt.

abgesaugt, mit 5%iger Salpetersäure, Wasser, Alkohol und Äther nacheinander saugend ausgewaschen und im Vak. über Schwefelsäure getrocknet.[20]

Für die Analyse[21] wurde aus 15%iger Salpetersäure umkristallisiert und im Wasserofen getrocknet:

13.3, 81.4 mg Subst. verb. 5.20, 31.42 ccm KCNS-Lösung, und diese entspr. 10.4, 62.84 mg Hg.

	Ber.	Hg	77.8%
Merkurofumarat			
	Gef.	„	78.2, 77.2%

Wenn man vorläufig zu einer Bromlaugelösung, die in einem aus Kochsalz und Eis bestehendem Kaltgemische abgekühlt ist, die in gleicher Weise abgekühlte Me-furfurallösung gibt und über Nacht im Eisschranke stehen lässt, so entsteht kein $CHBr_3$. Dieses Reaktionsgemisch wird mit verd. H_2SO_4 angesäuert, und einigemal mit Äther extrahiert. Die Ätherlösung wird verdampft, Alkohol zugegeben und Natriumbisulfit zugesetzt, um noch nicht reagiertes Me-furfural zu entfernen. Aus dem getrockneten Alkoholrückstand kamen alsbald Nadeln heraus, die aus Wasser umkristallisiert worden waren: Ausbeute 2.5 g aus 5.5 g Me-furfural. Mit Methylbrenzschleimsäure zeigt es keine Schmelzpunkterniedrigung

79.4, 95.3 mg	Subst. verb. gegen Phenolphthalein
6.16, 7.50 ccm	n/10-Natronlauge, während für Me-brenzschleimsäure
6.30, 7.56 ccm	berechnet werden.

(d) Bestimmung des Kohlensäuregas. —

Das aus dem Reaktionsgemische abgespaltete Kohlensäuregas wurde in n/10-Barytalösung eingeleitet und über ihrem aliquoten Teil mit 0.1 n-Salzsäure gegen Phenolphthalein als Indikator titriert,[22][23] wobei die Korrektion mit Na_2CO_3 vorgenommen wurde.[24] So wurden je 10 ccm 0.1 Mol. wässrige Lösungen von Me-furfural, Me-brenzschleimsäure, Furfural, Brenzschleimsäure und Ameisensäure in die Flasche hineingebracht, und es wurden 0.32 g Brom und 0.2 g Ätznatron enthaltendes, 10 ccm Wasser auf einmal hinzugefügt. Nach halbstündigem Stehen wurden KJ und verd. H_2SO_4 zugesetzt, und das entstandene Kohlensäuregas

wurde in die Barytalösung durch Strömung der kohlensäurefreien Luft hineingetrieben. Die dabei gefundenen Werte sind folgende:

Tablle III. CO_2-Abspaltung aus Me-furfural, Me-brenzschleimsäure, Furfural, Brenzschleimsäure und Ameisensäure.

Substanz mg	n/10-$Ba(OH)_2$ verb. ccm	CO_2 mg	CO_2 % *
Methylfurfural	5.89	12.96	29.5
Me-brenzschleimsäure	16.25	35.75	81.3
Furfural	7.12	15.66	35.6
Brenzschleimsäure	15.30	33.66	76.5
Ameisensäure	16.70	36.74	83.5

* Prozent der für 1 Mol. CO_2 aus 1 Mol. Subst. berechneten Menge.

Dass es keinen Unterschied zwischen Furfural und Me-furfural in Bezug auf oxydative Decarboxylierung durch Natriumhypobromit gibt, zeigt, dass die Einwirkung der Bromlauge nach der Kernöffnung in ganz ähnlicher Weise verlaufen ist. Man könnte nämlich denken, dass die Konfigurationen, wie sie über der Decarboxylierung einen Unterschied zeigen mögen, schon verloren waren—also die Furankerne sie gleichfalls haben. Weil die Ameisensäure durch gleiche Behandlung zu CO_2-Gas oxydiert wird, kann man noch nicht entscheiden, ob Ameisensäure aus einer Seite des den Kern geöffnetes Me-furfurals entsteht und dadurch CO_2-Gas sich abspaltet.

II. Tatsachen über die Öffnung des Furankerns bei Me-brenzschleimsäure

Zu je 50 ccm 20 milli Mol. wässriger Lösung von Me-brenzschleimsäure, welche aus Me-furfural durch Silberoxyd-Oxydation dargestellt und aus Wasser dreimal umkristallisiert wurde, werden n/50-Jodlösung und Alkali zugesetzt. Nach einstündigem Stehen oder Erhitzen auf dem Wasserbad unter Rückfluss wird mit verd. H_2SO_4 angesäuert und mit Thiosulfat zurücktitriert (I). Diese titrierten Lösungen werden zusammengegossen und in kongorotsaurer Lösung mit Äther ausgezogen. Die Ätherlösung wird abgedampft, und der ausgeschiedene Kristall wird aus Wasser umkristallisiert und getrocknet. Abermal wird diese Säure in 20 milli Mol wässriger Lösung jodometrisch titriert (II). Diese titrier-

ten Lösungen werden auch vereinigt und nach gleicher Weise noch einmal jodometrisch titriert (III).

Tabelle IV a. Jodverbrauch von Me-brenzschleimsäure.

Versuche Nr.	Bei 13°		Bei 98°	
	ccm	Atome	ccm	Atome
(I)	3.25	0.065	6.50	0.13
(II)	3.42	0.068	6.28	0.13
(III)	3.32	0.066	6.30	0.13

Tablle IV b.

Erhitzungsdauer	ccm	Atome
0	3.28	0.066
30 Min.	14.70	0.294
60 〃	31.25	0.625
100 〃	färbt sich rot	

Wie aus der obigen Tabelle erhellt, verbraucht Methylbrenzschleimsäure bei Zimmertemperatur 0.06 Atome und nach Erhitzen 0.13 Atome Jod. Dies zeigt, dass der Stoff, der 3 Atome Jod verbraucht hat, zu 2–4% gemischt ist, bzw. Methylbrenzschleimsäure in ihrer verd. wässrigen Lösung immer zu 2–4% in dem Kern öffnendem Zustande, sozusagen in einem Gleichgewicht vorhanden ist.

Wenn Methylbrenzschleimsäure auch in 20 milli Mol. enthaltender, 12%iger Salzsäurelösung unter Rückfluss auf dem kochenden Wasserbad während verschiedener Dauer erhitzt wird, danach je 50 ccm der Lösung pipetiert und mit n/50-Jodlösung und Alkali in obengenannter Weise jodometrisch titriert wird, so färbt sich diese, Methylbrenzschleimsäure enthaltende, Salzsäurelösung schon bei Erhitzung gelb bis orange (max. Absorp. $\lambda = 460\,m\mu$) und vermehrt den Jodverbrauch (Tabelle IV b), während die Brenzschleimsäure keine Erscheinungen zeigt.

Ich erlaube mir hiermit Herrn Professor M. Hamada für seine freundliche Anleitung und steten Ratschläge, die mir bei der Ausführung dieser und nachstehender Arbeit zuteil geworden sind, meinen herzlichsten Dank auszusprechen. Für die Möglichkeit zur weiteren Ausführung dieser Arbeit bin ich Herren Professoren Y. Oshima, K. Yamafuji und H. Iwata zu grossem Dank verpflichtet. Besten Dank schulde ich dem verstorbenen Herrn N.

Fujino, der mir unermüdliche Hilfe in dieser Untersuchung geleistet hat. Dieser Versuch wurde mit Hilfe der Ausgaben des Unterrichtsministeriums für Wissenschaftliche Forschung durchgeführt, wofür der Verfasser an dieser Stelle Dank sagen möchte.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

EIN DIMERES METHYLFURFURAL*

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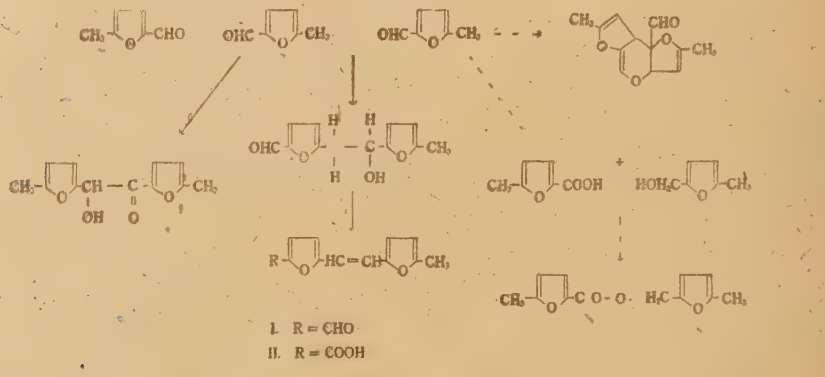
Während Verfasser die Einwirkung von Natriumhypojodit auf Methylfurfural verfolgte,[1] wurden neben dem harzartigen Hochpolymeren von Methylfurfural braunrote, chromsäureanhydridartige, glänzende Nadeln erhalten, welche, falls aus Alkohol und Wasser umkristallisiert wird, sich in orangefarbes, seidenartiges Kristall vom Schmp. 93.5° umwandelten.

Diese Substanz ist $C_{12}H_{10}O_3$ in ihrer Zusammensetzung, und enthält eine CH_3–C– und eine Carbonyl-gruppe. Die auffallendste Eigenschaft dieser Substanz ist ihre sehr intensive Halochromie,[2][3][4][5][6][7] nämlich sie löst sich mit hellroter Farbe in konzentrierten Mineralsäuren, verschwindet beim Verdünnen und färbt sich durch Zusetzen von Säure abermals. Die Carbonsäure vom Schmp. 165°, die aus dieser Substanz durch Oxydation mit Siberoxyd gewonnen wurde, zeigt keine Halochromie. Auf Grund dieser Eigenschaften nimmt man an, dass die Carbonylgruppe in der mit Doppelbindung konjugierten Stelle vorhanden sei, und dass diese Gruppe, genauer gesagt das Aldehyd, sich mit halochromer Eigenschaft beteiligt haben müsse. Diese Substanz und die von ihr abgeleitete Carbonsäure besitzen eine Doppelbindung ausser derselben des Furankerns, indem sie Permanganatlösung entfärben, Brom aufnehmen, sich mit Tetranitromethan färben

* Vorgetragen vor dem Bezirksverein West-Japan von der Agr. Chem. Soz. Japan mit Prof. M. Hamada gemeinsam am 7. Nov. 1947. Untersuchungen über Furfural, XIII. Mitteilung.

und bei der katalytischen Hydrierung 1 bzw. 5.5 Mol. Wasserstoff aufnehmen. Ausserdem haben sie keine Hydroxyl-, Methylenoxydgruppe usw. Dieser Kristall löst sich leicht in Alkohol, Aceton, Äther und Benzol, aber fast kaum in kaltem Wasser.

Diese Substanz könnte von dimerem Methylfurfural sein. Als dimeres Methylfurfural kann man sich leicht vorstellen, dass (a) benzoinartige Bindung, (b) Esterbindung zwischen Methylbrenzschleimsäure und Methylfurfurylalkohol, welche nach Cannizzaroscher Reaktion entstehen mögen, (c) Aldol- bzw. Croton-kondensation in welcher die Methylgruppe des einen Me-furfurals und die Aldehydgruppe des anderen teilhaft sind, und (d) Dien-Reaktion[8][9] usw., stattfinden werden.



Nun sind diese Substanz und Dimethylfuroin ganz verschiedenartig; das letztere wurde aus Me-furfural nach Fischers Verfahren[10] über die Furoindarstellung mit schlechter Ausbeute dargestellt. In der Esterbindung muss es zwei CH_3-C-Gruppen und darf es keine Aldehydgruppe geben, also ist diese nicht am Platze. Bei der Dien-Reaktion verhält es sich auch so. Gegen diese von vornherein unwahrscheinlichen Formulierungen wird die Verbindung mit einer Äthylenbindung (I) in Übereinstimmung damit als richtig erkannt, weil sie eine Doppelbindung, eine CH_3-C- und eine Aldehyd-gruppe enthielt, und doch die letztere mit konzentrierten Mineralsäuren jene Halochromie vorstellen wird.

Dass die Methylgruppe wie in Me-furfural, ohne eine Aktivgruppe in der Nachbarstelle zu haben, kondensieren kann, ist interessant.

Beschreibung der Versuche

I. Entstehung von [5-Methyl-furyl-(2)]-[5-formyl-furyl-(2)]-äthylen

Nachdem 11 g Me-furfural (0.1 Mol.) in 500 ccm Wasser gelöst und mit 500 ccm 0.4 n-Jodlösung und 25 g NaOH unter Schütteln gemischt wurden, wurde das Reaktionsgemisch durch Filtrieren von dem bald daraus ausscheidenden HCJ_3 entfernt und stehengelassen. Nach einigen Tagen fielen braunrote, glänzende Kristalle neben der harzigen Substanz aus, welch letztere sich im verd. Alkali bzw. Alkohol auflöst und beim Ansäuern bzw. Verdünnen wieder ausfällt.

Die gewonnene Masse wurde mit verd. Alkali und danach mit Wasser durchgewaschen und aus Alkohol umkristallisiert. Ausbeute ca. 0.4 g. Diese Verbindung kristallisiert in gelber, langer, seidenartiger Form vom Schmp. 93.5°, und zersetzt sich gegen 312° mit gelbem Qualme unter Sieden.

In der folgenden Tabelle werden die geeigneten Bedingungen für Entstehung dieser Verbindung dargestellt, und nebenbei wird gezeigt, dass keine Substanz aus Furfural gewonnen wurde. Diese Ausführung wurde im Masstabe eines Zehntels durchgeführt.

Tabelle 1.

	Alkalinität des Reaktionsgemisches	Jodverbrauch als 0.1 n	Beschaffenheiten von Lösung	Niederschlag	Kristall
Furfural	0.28 n	77.5 ccm	farblos	kein	kein
	0.52 〃	93.0 〃	gelb	〃	〃
	0.92 〃	114.2 〃	braun	〃	〃
	1.33 〃	117.5 〃	〃	〃	〃
	0.52 〃	ohne Zusetzen	dunkel	〃	〃
Me-furfural	0.06 〃	16.5 〃	farblos	〃	〃
	0.13 〃	17.0 〃	orangegelb	〃	〃
	0.28 〃	17.5 〃	hellrot	〃	weniger Nädelchen
	0.36 〃	22.5 〃	braun, trüb	〃	wenige Nadeln
	*0.52 〃	26.0 〃	rot, trüb	öliger, harziger	Nadel ca. 40 mg
	0.92 〃	55.0 〃	dunkel	harziger	wenige Nadeln
	1.33 〃	92.8 〃	〃	〃	kein

Diese Substanz ist neutral, zeigt durch die Trockendestillation mit CaO Fichtenspan-Reaktion, auch Molisch-Reaktion, und reagiert mit manchen Carbonylreagenzien in ihrer alkoholischen Lösung; sie reduziert ammoniakalisches Silbernitrat und Fehlingsche Lösung, allein eine Fuchsinschwefligesäurelösung nicht, und die auffallendsten Eigenschaften bestehen darin, dass sie sich mit Phloroglucin-Salzsäure hellblau, mit Hippursäure rot färbt, und ein Tropfen der letztern konzentrierte Schwefelsäure violett färbt, schliesslich dass sie Halochromie zeigt.

Dieser Kristall ist in Alkohol, Aceton, Benzol, insb. Äther leicht löslich, aber fast nicht in Wasser.

3.613 mg Subst.: 9.175 mg CO_2, 1.660 mg H_2O
$C_{12}H_{10}O_3$ Ber. C 71.11 H 4.99
Gef. „ 69.26 „ 5.14

Molekülargewichtsbestimmung nach Rast:

0.258 mg Subst. in 3.122 mg Kampfer
(K=39), $\Delta T = 15.7°$ Gef. 205
Ber. 202
0.305 mg Subst. in 3.496 mg Kamfer
(K=39), $\Delta T = 16.7°$ Gef. 203

Carbonylgruppebestimmung nach E. v. Meyer:

mg Subst	ccm der dem Hydrazon entsprechenden 0.02 n-Jodlösung	Gefundene Prozentgehalte an Aldenhyd	Zahl der Ald.-Gruppe
9.081	2.560	15.23	1.1
7.678	2.362	16.63	1.2
1.320	0.322	13.16	0.95
Ber.		13.86	1.00

Katalytische Hydrierungen: Die Versuch wurden in der üblichen Anordung durchgeführt. Pt- und Pd-Schwarz wurden nach Vorschrift von Willstätter und Waldschmidt-Leitz hergestellt. Als Lösungsmittel wurden Eisessig und Essigester benutzt. Die aufgenommene Menge Wasserstoff, die Hydrierungsdauer sowie die angewandte Substanz- und Katalysator-menge werden in der folgenden Tafel gegeben.

mg Subst.	mg Katalysator	ccm Lösungsmittel	ccm H_2 bei N.T.P.	Absorbierungsdauer in Min.	Mol. H_2
50.5	Pd 50	Eisessig 10	6.05	25	1.08
"	"	Essigester 10	30.80	60	5.50
"	Pt 50	Eisessig 10	5.88	30	1.50
"	"	Essigester 10	31.60	60	5.64

Falls Eisessig als Lösungsmittel benutzt wurde, wurde 1 Mol. Wasserstoff aufgenommen; während 5.5 Mol. Wasserstoff bei Benutzung von Essigester absorbiert wurden. Daher kann man sich leicht vorstellen, dass eine Äthylenbindung zwischen zwei Furankernen dem ersteren 1 Mol. zugesetzt hätte, und alle Doppelbindungen und eine Carbonylgruppe in dem letzteren eine unregelmässige Wasserstoffaufnahme vollzogen hätte. Am Ende der Hydrierung wurde die Flüssigkeit vom Katalysator abfiltriert und im Vak. eingedampft. Der kristalline Rückstand im Fall des Eisessigs liess sich aus Alkohol umkristallisieren: Braungelbe Nadeln vom Schmp. 78°. Der Rückstand vom anderen Lösungsmittel ist farbols und ölig. (Darüber wurden noch keine genaueren Untersuchungen angestellt.)

Oxydation mit Chromsäure: Diese Verbindung, die von ihr abgeleitete Carbonsäure und 5.5'-Dimethylfuroin liefern beim Erhitzen nach Vorschrift[11] mit 5n-Chromsäure in schwefelsaurer Lösung 0.92, 0.95 und 1.90 Mol. flüchtige Säure.

Subst.	Angewendetes Gewicht (mg)	Verbrauchte ccm 0.01n-NaOH (F=0.9837)	Essigsäure	
			mg	Mol.
Subst. (I)	11.602	5.361	3.166	0.91
"	9.811	4.653	2.748	0.93
Subst. (II)	4.502	2.010	1.187	0.95
5.5'-Dime-furoin	14.004	12.305	7.266	1.90

Die Natriumsalze dieser Säuren wurden durch ihren Schmelzpunkt mit Natriumacetat identifiziert. Es wurde somit von der $CH_3-C(-CH-)(-O-)$ Gruppe in diesen Verbindungen in sehr guter Ausbeute Essigsäure gebildet. Wenn $CH_3-C(-CH=)(-O-)$ im Molekül

vorhanden wäre, so würde seine Zahl nur 1 sein, und es würde fast quantitativ zur Essigsäure zerfallen.

II. [5-Methyl-furfuryliden]-Mthylbrenzschleimsäure

5 g Silberoxyd wurden in einer Lösung von 0.8 g Natriumhydroxyd in 50 ccm Wasser suspendiert, 0.3 g der oben beschriebenen Substanz zugegeben und eine halbe Stunde unter Rückfluss gekocht. Nach Abkühlung wurde die Lösung vom Silber filtriert, mit Schwefelsäure angesäuert und mit Äther ausgezogen. Nach Abdampfen des Äthers wurde die Kristallmasse aus Alkohol umkristallisiert: gelbe Blätter vom Schmp. 165°. Ausbeute ca. 0.2 g. Sie sind leicht löslich in Alkohol, Äther, schwerer in Wasser.

Die Verbindung erwies sich in ihrem Verhalten gegen Brom und $KMnO_4$ (Aceton) als ungesättigt, zeigte aber gegen konz. Mineralsäuren keine Halochromie.

Neutralisationsäquivalent:

mg Subst.	ccm 0.01 n-NaOH	Äquivalent
5.695	2.676	212.8
13.065	12.039	216.5
8.504	3.991	213.1

III. 5,5′-Dimethyl-(β-oxy-α-oxo-α, β-di-α-furyl äthan) od. 5,5′-Dimethylfuroin

22 g Me-furfural wurden mit 20 g Alkohol gemischt, mit einer Lösung von 2 g Kaliumcyanid in 40 ccm Wasser versetzt und eine halbe Stunde unter Rückfluss gekocht. Nach Eindampfen der Flüssigkeit zu ca. 30 ccm wurde sie auf eine Tonplatte gestrichen und aus Alkohol umkristallisiert: gelbe Prismen vom Schmp. 164°, Ausbeute ca. 2 g.

In manchen Eigenschaften ist dies Kristall dem Furoin ähnlich; 5.5′-Dimethylfuroin löst sich schwer in kaltem Wasser, leichter in warmem Alkohol, und in kalter konzentrierter Schwefelsäure mit blaugrüner Farbe. Es löst sich auch leicht in verdünnter wässrig-alkoholischer Alkalilauge zu einer violetten Flüssigkeit, die an der Luft unter Bildung von 5,5′-Dimethylfuril entfärbt wird, wie zu erwarten ist.

Herrn Prof. M. Hamada bin ich für die Leitung meiner Versuche zu grossem Dank verpflichtet.

Dieser Versuch wurde mit Hilfe der Ausgaben des Unterrichtsministeriums für Wissenschaftliche Forschung durchgeführt, wofür der Verfasser an dieser Stelle Dank sagen möchte.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

THE AMINO ACIDS OF THE SERICIN FRACTIONS OF SILK

Shuiku Sasaki and Masato Miyauchi

Raw silk consists of two proteins, inner fibrous core "fibroin" and sheath "sericin". The sericin is easily removed from the fibroin with boiling water. Recent literature (1) (2) (3) (4) (5) states that the sericin extracted from raw silk is a mixture of, at least, two simpler proteins showing different behaviors, such as solubilities, their resistance to proteolytic enzymes, and their action in electric dialysis. Although it is probable that the greater part of these different properties is produced by the drustic action at the extraction of sericin and during the aging of raw silk, whether the sericin in the raw silk, after spun by silk worm, is simple homogeneous protein or a mixture of simpler proteins has not been accertained. For example, Rutherford and Harris (5) stated that sericin B is gradually converted into sericin A during the extraction of raw silk in an autoclave at 114–115°, and the contents of tyrosin in sericin A and sericin B prepared from the raw silk extracted for a short time with hot water are apparently different: Mosher (2) stated that sericin B is slowly converted into sericin A by aging.

Many studies (6) (7) (8) (9) concerning the amino acids of the hydrolysate of the sericin were reported, but most of the previous studies were carried out by the old ester method or with several limited amino acids, and, thus far, only 50% of the sericin has been accounted for in the yields of amino acids isolated.

Since it was our purpose to determine the amino acid content in the sericin, and, if possible, to compare the difference of

chemical composition of sericin fractions, we exployed recently developed methods for the isolation of amino acids. We recovered about 65% of the total nitrogen from sericin B and sericin A respectively as crystalline amino acids or their derivatives except ammonia and triptophane, and found no appreciable difference of amino acids content between these fractions which had different physical properties. Besides our experiment, Abderhalden and Zumstein (8) found norvaline, chitosamine and glucuronic acid, and Alders (10) found cystine from the unfractionated sericin.

The figures of amino acids nitrogen of the sericin summed up in the above experiments does not exceed 70%. We cannot understand the causes of this low value, since one of our writers, applying almost the same procedures, recovered about 80% amino acids from the proteins of soybean (*Soja hisoida*) (13), and ryokuto (*Phaseolus Mungo* L. var *radiatus* Bak) (14) respectively.

Experimental

The Preparation of Sericin Fractions from Cocoons

Because of the differences in the nature of silk from different varieties of worms and in the age of the silk, we prepared our sericin from cocoons known as "Gunze Blue" recently spun in laboratory.

200 g. of sericin was extracted two times successively with 21 of water for one half hour at a pressure of 15 lbs. in an autoclave. The extract was acidified with acetic acid to pH 4.0. The precipitate, sericin B, was filtered, and washed well with water, alcohol and ether successively. The aqueous filtrate and the washing from sericin B were poured into 4 volumes of 95% alcohol. The precipitate, sericin A, was filtered, and washed with alcohol and ether. The yield of sericin B was 13.3%, and that of sericin A was 8.9% respectively from the cocoons as moisture free bases.

Table 1.

	Moisture	Ash	Nitrogen (ash and moisture free bases)
Sericin B	5.5	0.5	16.3
Sericin A	6.0	1.8	16.5

Sericin B precipitated in the bottom of a beaker as a white powder and sericin A aggregated in the liquid as a somewhat fibrous flocculence. The ash content of sericin A was reduced to 1.1% when this precipitate had been dissolved in 0.2% NaOH solution, dialyzed, and reprecipitated with alcohol.

Isolation of Amino Acids from the Hydrolysate of Sericin

The sericin was hydrolyzed for 25 hours with 20% HCl or 25% H_2SO_4. We selected either HCl or H_2SO_4 in accordance with the method of isolation of individual amino acid. When monoamino monocarboxylic acids were isolated by the ester method, basic amino acids had been previously removed with phosphatungstic acid from the hydrolysate of the sericin, dicarboxylic acids removed as barium salts according to the method of Jones and Moeller (12), and then the esters of monoamino monocarboxylic acids were prepared according to the method of Forman (15).

Ammonia.——Ammonia was volumetrically estimated by the usual method from the distillate of the weak alkaline solution of the hydrolysate of the sericin.

Glycine.——Two methods of isolation were used. (A). The hydrolysate of the sericin was esterified as usual. The distillate of lower boiling point (temperature of vapor up to 70° at 16 mm pressure) of esters was hydrolyzed with boiling water and converted into cupper salts. From the water soluble and methanol insoluble cupper salts (16) glycine was isolated as picrate according to the method of Levene and van Slyke (17). (B). Glycine was directly crystallized as glycine potassium trioxalatochromate according to the method of Bergmann and Fox (18).

Alanine.——Two methods of isolation were used. (A). Alanine was isolated from the filtrate of the glycine picrate (17). (B). Alanine was directly crystallized as alanine dioxypyridate according to the method of Bergmann (19).

Valine.——Valine was isolated from the combined hydrolysates of esters of lower and higher boiling point (temperatures of vapor up to 70° at 16 mm pressure and up to 70° at 3 mm pressure) purifying as methanol soluble cupper salt (16) and ammoniacal water soluble lead salt (20) successively.

Leucine.——Leucine was isolated from the hydrolysate of esters

of higher boiling point purifying as water insoluble cupper salt (16) and ammoniacal water insoluble lead salt (20) successively.

Isoleucine.——Isoleucin was not detected. If it was present, it would be isolated from the leucine fraction as its methanol soluble cupper salt and ammoniacal water insoluble lead salt.

Phenylalanine.——Phenylalanine was not detected. If it was present, it would be isolated as its hydrochloride from the distillation residue of the esters.

Serine.——Serine was isolated as water soluble cupper salt from the unesterified residue according to the ester method of Foreman (15).

Proline and Oxyproline.——These amino acids were detected neither by the method of Town (21) nor by the method of Bergmann (22).

Arginine.——Arginine was isolated as flavianate according to the micromethod of Vickery (23), of which we has proved to obtain most accurate result.

Histidine and Lysine.——These amino acids were estimated from the filtrate of the arginine flavianate according to the micromethod of Tristram (24).

Tyrosine.——Tyrosine was recovered in a free state from the several fractions of Foreman's ester method (15).

Aspartic Acid and Glutamic Acid.——These amino acids were isolated according to the method of Jones and Moeller (12).

Oxyglutamic acid.——Oxyglutamic acid was not detected according to the method of Dakin (25).

Tryptophane.——Tryptophane was colorimetrically determined according to the method of Folin and Marenzi (28).

Table 2.

	% of weight of protein		% of total nitrogen	
	Sericin B	Sericin A	Sericin B	Sericin A
Ammonia	2.2	2.0	11.1	10.0
Glycine	4.1 (3.2)*	3.8 (3.0)*	4.7	4.3
Alanine	11.9 (10.6)*	12.0 (9.4)*	11.5	11.4
Valine	1.3	0.9	1.0	0.7
Leucine	1.7	1.4	1.1	0.9
Isoleucine	none	none	none	none
Phenylalanine	none	none	none	none
Serine	13.5	12.6	11.1	10.2
Proline	none	none	none	none

Oxyproline	none	none	none	none
Arginine	5.1	5.1	10.0	10.0
Histidine	1.1	1.3	1.8	2.1
Lysine	1.2	1.1	1.4	1.3
Tyrosine	5.2	4.9	2.5	2.3
Aspartic acid	13.8	13.0	8.9	8.3
Glutamic acid	2.9	2.4	1.7	1.4
Oxyglutamic acid	none	none	none	none
Tryptophane	1.0	1.1	0.8	0.9
Total	65.0	61.6	67.6	63.8

* These figures are the results of ester method.

In addition to the above figures sericin contains 1.25% of norvalin (8), 0.5% of chitosamine (8), 0.6% of glucuronic acid (8) and 1.04% of cystine (10).

Summary

Sericin, one of the proteineous constituents of raw silk, was extracted with water in an autoclave at a pressure of 15 lbs., and divided into two fractions, sericin B and Sericin A. The former was insoluble in water at pH 4.0; the latter was soluble in water at pH 4.0, but insoluble in concentrated alcohol. Although these two fractions were different in appearance and physical properties, they seemed to the identical with amino acids content within the limit of experimental errors.

Recovery of total nitrogen as amino acid was about 70%.

The authors are indebted to the Sanshi Chōsakai (Association of the sericultural research) for a generous subsidy.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

STUDIEN ÜBER DIE KRISTALLISIERTE KATALASE (IV)

Molekulargestalt und Molekulargewicht

Masaharu Shirakawa

Früher ermittelte Stern[1] die Diffusionskonstante von Leberkatalase (Pferde, Rinder und Schweine) nach der Northropschen Glasmembranmethode[2] und berechnete daraus ein Molekulargewicht von 61,900 für Katalase. Aus seinen weiteren Versuchen[3,4] über die Sedimentationskonstante bei Ultrazentrifugierung, schloss er aber auf ein Molekulargewicht von 250,000 bis 300,000 für dasselben Ferment. Sumner und Gralén[5] entschieden einmal ein Molekulargewicht von 248,000 für die nach ihrer eigenen Methode[6] kristallisierten Leberkatalase auf Grund von Sedimentations- und Diffusionsbestimmungen[7], was aber später zu 225,000 korrigiert wurde[8]. Die früheren Versuche von Zeile[9], Diffusionskonstante der Katalase durch Glasmembranmethode zu bestimmen, waren ihm dadurch missglückt, dass die Katalase durch Passierung der Membran stark inaktiviert wurde. Stern[1] zeigte später, dass die von Zeile beobachtete Inaktivierung der Katalase durch Regulierung von pH der Enzymlösung vermeidet werden kann; z. B. bei pH 7.0 bis 7.2 wurde keine Inaktivierung mehr beobachtet. Das von Stern zuerst ermittelte Molekulargewicht der Katalase, 68,000 sieht allzu klein gegenüber dem Wert, 225,000 nach Sumner und Gralén aus, und es scheint dem Verfasser, dass Stern selbst die Ungenauigkeit seiner Bestimmung des Molekulargewichtes anerkannt haben sollte, weil er im späteren Bericht über das Molekulargewicht desselben Fermentes keinen früher ermittelten Wert anführte. Es kann daher angenommen werden, dass unter den vorgeschlagenen Mole-

kulargewichten der Katalase der Wert von 225,000 nach Sumner und Gralén am vertrauenswürdigsten sei.

Zur Bestimmung der Diffusionskonstante ist die Glasmembranmethode von Northrop im Vergleich mit der häufig angewandten Methode nach Lamm beträchtlich vorteilhaft dadurch, dass eine einfachere Einrichtung und kürzere Bestimmungszeit genügen, und dass bei Behandlung der biowirksamen Substanzen, wie Fermenten, ihre Diffusionsgeschwindigkeit mittels der biochemischen Wirkungen bestimmt werden kann. Infolge mancher theoretischen und praktischen Prüfungen hat man neuerdings eine erfolgreiche Anwendbarkeit dieser Methode gefunden.[10]

Die Genauigkeit des von uns selbst hergestellten Diffusionsapparates nach Northrop wurde vorher für Carboxyhämoglobin bestätigt und dann die Diffusionskonstante der kristallisierten Leberkatalase, welche nach der in letzterem Berichte[11] erwähnten Methode isoliert und umkristallisiert wurde, liess sich bestimmen. Daraus ergab sich eine Diffusionskonstante von 3.34×10^{-7} cm^2/Sek. bei 10°C. Rechnet man diesen Wert auf 20°C um, so wird 4.4×10^{-7} gewonnen, was mit dem früher von Sumner vorgeschlagenen Werte, 4.5×10^{-7}, in guter Übereinstimmung steht. Angenommen, dass Katalasemoleküle monodispers und kugelförmig sich in der Lösung befinden, kann ein Molekulargewicht von 357,000 leicht aus der Diffusionskonstante berechnet werden. Diese Annahme ist aber nicht ganz richtig, weil das Viskositätsverhalten der Katalaselösung, wie später erwähnt, einen deutlichen Charakter von nichtkugelförmigen Teilchen zeigte.

Die Theorien über die Beziehung zwischen Reibungsverhalten und Gestalten kolloider Teilchen, wie Kohlenhydrate und Eiweissstoffe, entwickelten sich neuerdings auffallend, und dadurch ist häufig die Nichtkugelförmigkeit von Gestalten verschiedener Proteine diskutiert worden[12].

Aus der gefundenen Diffusionskonstante und den Viskositätsdaten kann man nach Polson[12] leicht sowohl das Achsenverhältnis als das Molekulargewicht eines kolloiden ellipsoiden Teilchens berechnen. Der Verfasser gewann einen Wert von 3.4 für das Achsenverhältnis des als ein ellipsoides Teilchen gedachten Katalasemoleküls und dann mit Rücksicht auf die Diffusionskonstante ein Molekulargewicht von 249,900 und 252,800 für längliche und abgeplattete ellipsoide Teilchen beziehungsweise. Es ist heute

noch nicht zu sagen, ob eine längliche oder abgeplattete Gestalt für das Katalasemolekül gelten solle.

I. Bestimmung von Diffusionskonstante

1) Apparatur und Ausführung.

Der Glasfilter (Jena G_4), dessen Filterplatte mit Schmirgelstein bis zur Dicke von 0.6 bis 0.8 mm dünn gehobelt wurde, kam zur Verwendung. Die apparativen Einzelheiten gehen aus Abb. 1 hervor. Bei der Bestimmung lässt sich die zu versuchende Fermentlösung in die innere Kammer (b) einsaugen. Wenn die in der Glasmembran (a) eingesperrten Blasen völlig vertreibt werden, schliesst man den Hahn (d), wäscht die äussere Wand der inneren Kammer schnell mit Lösungsmittel und wischt weiter sorgfältig mit Filterpapier ab. Die innere Kammer wird dann in dem im Thermostat festgehaltenen äusseren Zylinder (f), der mit 25 ccm von demselben Lösungsmittel beschickt war, in der Weise vorgerichtet, wie die Glassmembran 1 bis 2 mm unter dem Maniskus zum Eintauchen kommt. Gleich mit Eintauchen beginnt Diffusion und hört durch Abreissen auf. Es ist dabei sehr wichtig, die Glasmembran wasserrecht und fest zu halten. Während dieses Verfahren anscheinend ziemlich grob für Bestimmung von Diffusionskonstante aussieht, kann man jedoch durch sorgfältige Übungen mit Verbindungen von bekannten Diffusionskonstanten genügend genaue Ergebnisse erreichen. Das äussere Lösungsmittel dient zur Analyse. Alle Bestimmungen wurden bei 10°C ausgeführt.

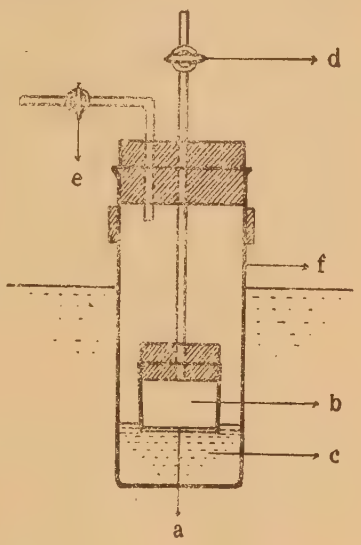

Abb. 1. Diffusionsapparat.

2) Eichung der Membran mit N/10-HCl.

Um eine normale Diffusionskonstante zu gewinnen, hatten Northrop und Anson die von Öholm[13] ermittelten Daten für N/10-

HCl auf 5°C extrapoliert und dann einen Wert von 1.85 cm^2/Tag. erreicht. Wie auch von Zeile schon erwähnt wurde, ist diese Extrapolierung unrichtig. Als eine normale Diffusionkonstante nahm der Verfasser den folgenden Wert nach James und Gorden[14] auf:

$$D_{10}\text{-(N/10-HCl)} = 2.03 \times 10^{-5}\ cm^2/Sek.$$

Nach zweistündiger Vordiffusion von 0.104 N-HCl gegen CO_2-freies Wasser, wodurch die HCl-Konzentration der inneren Kammer auf etwa 0.100 N sank und weiter ein bestimmtes Diffusionsgleichgewicht in der Membran sich erreichen liess, wurde der äussere Zylinder durch einen frischen ersetzt. Die diffundierte HCl-Menge wurde stundenweise titrimetrisch bestimmt und in Kubikzentimeter der entsprechenden Mutterlösung (Q_{ccm}) ausgedrückt. Bei nacheinander folgenden Messungen, wurde die Konzentrationsabnahme der Mutterlösung mit Rücksicht auf die dabei diffundierte HCl-Menge korrigiert. Ergebnisse der Versuche zeigt Tabelle I.

Tabelle I. Bestimmung der Membrankonstante mit 0.1N-HCl bei 10°C.

Diffusionszeit (Min.)	0.01 N-NaOH (ccm)	Q (ccm)
60	1.90	0.190
60	1.88	0.189
60	1.88	0.191
	Mittelwert	0.190

Die zu suchende Membrankonstante (K) lässt sich nach folgender Formel berechnen:

$$D = \frac{K\,G}{t},$$

darin ist: $D = 2.03 \times 10^{-5}\ cm^2$/Sek., $Q = 1.90$ ccm und $t = 60 \times 60$ Sek. Daraus geht hervor, $K = 0.385\ cm.^{-1}$

3) Bestimmung von Diffusionskonstante des Carboxyhämoglobin.

Zur Mikroanalyse einer äusserst kleinen Menge des diffundierten Proteins wie Carboxyhämoglobin oder Katalase dient die nephelometrische Methode nach Franceschetti und Wieland.[15] Fünf ccm Probenlösung wurde mit 5 ccm von 5% HCl und 10 ccm von 10% Na-sulfosalycylatlösung versetzt und die dabei entstandene Trübung nephelometrisch bestimmt. Zur Standardtrübung wurde eine Suspension angewant, welche dadurch hergestellt wurde, dass sich

eine geeignete Proteinlösung von bekannter Konzentration in ganz gleicher Weise behandeln liess. Nach dieser Methode konnten wir 0.1 mg Protein mit Fehler kleiner als ± 1.5% bestimmen; dieser Fehler ist aber beträchtlich stärker im Vergleich mit 0.2% von Franceschetti, was auf der Unvollkommenheit von Nephelometer beruht. Bei dieser Gelegenheit kann man sich jedoch mit der Genauigkeit dieses Grades begnügen, weil die Proteinanalyse nichts anders als ein Hilfsmittel für Diffusionsbestimmung ist.

Eine 2%ige Lösung des Carboxyhämoglobins in physiologischer Salzlösung, welches nach Heiderberger[16] aus Rinderblut hergestellt wurde, war an Diffusionsversuche gestellt worden. Zur Standardlösung bei Nephelometrie diente eine verdünnte Lösung (1:500) von 2%iger Lösung des Carboxyhämoglobins. Tebelle II zeigte die Ergebnisse.

Tebelle II. Bestimmung von Diffusionskonstante des Carboxyhämoglobins in 0.85 %iger NaCl-Lösung bei 10°C (nach Nephelometrie).

Diffusionszeit (Stunden)	Diffundierte Menge (mg/25 ccm)	Q (ccm)
10	1.03	0.0538
10	1.08	0.0540
10	1.06	0.0538
	Mittelwert	0.0538

Für die Diffusionskonstante einer Substanz von bekannten K und Q, wie obenerwähnt, gilt die folgende Formel:

$$D_{10°} = \frac{K\,Q}{t},$$

wobei K = 0.385, Q = 0.0538 und t = 10/24 Tag. bedeutet. Daraus ergibt sich, D_{10} = 0.0496 cm^2/Tag. Korrigiert diesen Wert für reines Wasser, so kann man 0.0502 cm^2/Tag. an Stelle von 0.0496 erreichen. Wenn man hiervon das Molekulargewicht des als kugelförmig gedachten Carboxyhämoglobins, wie allgemein angenommen wird, nach Einsteinscher Formel berechnet, so gewinnt man 68,800, was mit 68,000 nach Northrop und Anson, 68,600 nach Zeile, 68,700 nach Stern und 69,000 nach Svedberg[17] gut übereinstimmt.

4) Bestimmung der Diffusionskonstante der Katalase.

Die Katalase, die durch viermalige Umkristallisierung und mehrmaliges Waschen mit M/20-Phosphatpuffer (pH 7.0) gereinigt

wurde, wurde in derselben Pufferlösung bis zur Sättigung aufgelöst. Es ist nötig hierbei zu bemerken, dass die Katalase, mehrere Stunden bei 10°C stehen gelassen, mehr oder weniger inaktiviert wird, besonders stark in verdünnter Lösung. Bei der Diffusion durch die Membran ist das gerade der Fall. Daher muss man in diesem Falle diese Inaktivierung in Berücksichtigung ziehen.

Unter diesen Bedingungen steht die diffundierte Menge von Katalase (Q) mit Diffusionszeit (t) im linearen Verhältnis. Dies macht es möglich, den Grad der auf Diffusion beruhenden Inaktivierung zu ermitteln. Zur Bestimmung der diffundierten Mengen von Katalase dienten die Aktivitätsbestimmung der Katalase und auch Proteinbestimmung nach Nephelometrie. Tabelle III zeigt die gesamten Ergebnisse, und in Abb. 2 wird die Beziehung zwischen Q und t dargestellt.

Tabelle III. Diffundierte Mengen der Katalase ausgedrückt in der katalatischen Wirkung und Proteinmenge (10°C).
Kat. f. = 29,000, Katalasekonz. = 0.975%, k/ccm = 224)

Diffusionszeit (Stunden)	Diffundierte Mengen der Katalase			
	Katalasewirkung		Protein	
	k/5 ccm*	Q ccm	mg Prot./5 ccm	Q ccm
1.0	0.0844	0.00189	—	—
2.0	0.260	0.00580	—	—
3.0	0.408	0.00911	—	—
5.0	0.676	0.0150	—	—
8.0	1.05	0.0235	—	—
11.0	1.30	0.0290	0.0605	0.0311
14.5	1.47	0.0328	0.0850	0.0395
18.0	1.60	0.0356	0.0940	0.0477

*) k: Die Geschwindigkeitskonstante der monomolekularen Reaktion, in welcher Katalase beteiligt.

Es geht aus Abb. 2 hervor, dass bei mehr als 8 stündiger Diffusion die Inaktivierung der Katalase allmählich mit steigender Versuchszeitdauer(t) zunimmt. Der Q-Wert aus Proteinbestimmung ist dagegen zu der Zeitdauer annähernd proportional. Innerhalb 8 Stunden stimmen die Ergebnisse aus beiden Methoden gut überein. Der Verfasser wählte daher dreistündige Diffusionszeit aus. Die Ergebnisse des Diffusionsversuches bei 10°C über Katalase wurden in Tabelle IV gezeigt.

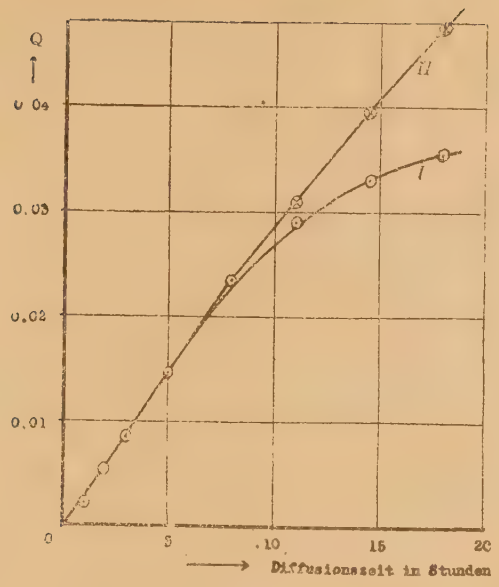

Abb. 2.

I: ausgedrückt in Katalasewirkung.
II: ausgedrückt in Proteinmenge.

Tabelle IV. Diffusionsgeschwindigkeit der Katalase für M/20-Phosphatpuffer (pH 7.0) bei 10°C (Kat. f. = 31,000).

Konz. der Katalase (%)	Diffusionszeit (Stunden)	Diffundierte Menge (k/5 ccm)	Q (ccm)
0.765	3.0	0.457	0.00933
	3.0	0.449	0.00916
	3.0	0.452	0.00922
0.153	3.0	0.0880	0.00906
	3.0	0.0890	0.00916
	3.0	0.0886	0.00912
		Mittelwert	0.00918

II. Bestimmung von Reibungskonstante

Die bestimmung von Reibungskonstante wurde nach Ostwaldscher Viskosimetrie bei 10°C für Kataloselösungen ausgeführt, welche 0.25 bis 1.00 %ige Katalase in M/20-Phosphatpuffer (pH 7.0) enthielten. Die in Tabelle V angeführten Werte von t und s sind Mittelwerte bei dreimaligen Messungen.

Tabelle V. Reibungsmessungen von Kataläselösungen bei 10°C.

Konz. der Katalase (%)	t (Sekunden)	s	$\frac{\eta}{\eta_0}$	ln A
0	130.7	5.0772	1.0000	—
1.00	135.0	5.1062	1.0386	0.0380
0.75	133.9	5.0990	1.0285	0.0375
0.50	132.8	5.0915	1.0191	0.0380
0.25	131.7	5.0843	1.0091	0.0380
			Mittelwert	0.0378

Hierin bedeutet t die Zeit, welche die Lösung verbraucht, um von der oberen Markierung bis an die untere des Apparates auszufliessen, s das Gewicht der Lösung, im Piknometer eingenommen, η/η_0 die relative Reibungskonstante, ln A relative Reibungskonstante bei unendlicher Verdünnung nach Polson.[14]

III. Bestimmung des Spezifisches Partialvolumens von Katalase

Um die Molekulargestalt aus Reibungsversuchen zu erhalten, soll man zuvor das spezifische Partialvolumen bestimmen, welches das von Katalase eingenommenen Volumen in Volumeneinheit der Lösung bedeutet. Zur Zwecke diente die Piknometrie nach folgender Formel:

$$d_0 + s - V \cdot s \cdot d_0 = d_1.$$

Hierbei ist, d_0 = die Dichte des Lösungsmittels, d_1 = die Dichte der Katalaselösung, s = das Gewicht der in 1 ccm Lösungsmittel aufgelösten Substanz und V = das spezifische Partialvolumen.

Diese Formel kann auf Grund von der folgender Tatsache abgeleitet werden. Versetzt man 1 ccm Lösungsmittel mit s g Substanzen, so nimmt das Volumen um s·V ccm zu, und beträgt das gesamte Gewicht $(d_0 + s)$ g. Daher soll das Gewicht von 1 ccm Lösung, welche s g Substanzen in Volumeneinheit enthält, nämlich d_1 g, dem Wert von $(d_0 + s - s \cdot V \cdot d_0)$ g gleich stehen, worin $s \cdot V \cdot d_0$ das Gewicht desjenigen Lösungsmittels bedeutet, welches mit dem zugenommenen Volumen korrespondiert.

Die Gefundenen Werte bei 10°C für 1.65 % ige Katalaselösung M/20-Phosphatpuffer (pH 7.0) sind die folgenden: $d_0 = 1.0075$, $d_1 = 1.0117$, $s = 0.0165$. Daraus ergibt sich

$$V = (d_0 + s - d_1)/(s \cdot d_0) = 0.740.$$

IV. Berechnungen

1) Molekulargewicht aus Diffusionskonstante.

Nach Einsteinscher Diffusionsgleichung,

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi\eta r}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

kann man aus den bekannten Werten von Diffusionskonstante (D) und Reibungskonstante (η) des Lösungsmittels bei T° den Radius (r) des kugelförmigen Moleküls berechnen. Unter Berücksichtigung des spezifischen Gewichtes der gelösten Substanz ergibt sich weiter das Molekulargewicht.

Über die Katalaselösung sind folgende Werte bekannt worden. $D = 0.0289$ cm²/Tag. od. 0.3346×10^{-6} cm²/Sek., $\eta = 0.0136$ g/cm. Sek., $R = 8.3 \times 10^{-7}$ Erg/Grad.Mol., $N = 6.06 \times 10^{23}$, $T = 283.1$. Darrus errechnet sich der Radius des als kugelförmig gedachten Katalasemoleküls, wie folgt:

$$r = 4.706 \times 10^{-7} \text{ cm.}$$

Für das Molekulargewicht gilt

$$M = (4/3)\pi r^3 g N, \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

darin bedeutet g das spezifische Gewicht der Katalase, welches in erster Näherung mit der reziproken Zahl von dem spezifischen Volumen (V) übereinstimmt. Es ergibt sich daraus: M = 357,000.

2) Achsenverhältnis aus Reibungskonstante.

Einstein war der erste, der eine hydrodynamische Beziehung zwischen dem Volumen der in Lösung befindlichen Substanz und der Viskosität der Lösung ableitet. Für starre sphärische Teilchen gab er die folgende Formel an:

$$\frac{\eta}{\eta_0} = 1 + 2.5\,G,$$

worin η/η_0 das Verhältnis zwischen der Viskosität der Lösung und der des Lösungsmittels bedeutet; G ist das Volumen gelöster Substanz in der Volumeneinheit der Lösung. Die obenerwähnte Formel gilt allerdings nicht für Teilchen, deren Gestalt von der Kugel abweicht.

Wo. Ostwald[18], W. Kuhn[19] u.a. wiesen darauf hin, dass auch der Einfluss der Brownsche Bewegung dem orientierenden Einfluss der hydrodynamischen Kräfte entgegensteht. Wenn die Brownsche

Bewegung so stark ist, dass eine dauernde isotrope Anordnung der Teilchen zu erwarten ist, gilt nach Kuhn die Formel:

$$\frac{\eta}{\eta_0} = 1 + 2.5\,G + \frac{G}{16}\left(\frac{b}{a}\right)^2, \quad \ldots\ldots\ldots\ldots (3)$$

wobei b/a das Verhältnis der langen Achse eines Ellipsoids zur kurzen bedeutet. Bei der Behandlung von Proteinlösungen soll G das hydrierte Molekularvolumen bedeuten, das die "nicht-hydrierte" Proteinmoleküle in 1 ccm von ihrer 1%igen Lösung einnehmen.

Für die Beziehung der Reibungskonstante zur Konzentration gilt eine praktische und häufiger angewandte Formel, die von Arrhenius empirisch abgeleitet wurde: $\eta/\eta_0 = A^c$, hierin ist A eine Konstante und c die Konzentration der in der Lösung befindlichen Substanz. Die Anwendbarkeit dieser Formel auf Proteinlösungen wurde von Loeb[20] und später Haugaard und Johnson[21] gezeigt. Bei der Berechnung der Gestalt von Proteinmolekülen wurde η/η_0 bei unendlicher Verdünnung benützt, wo die Teilchen nicht gegenseitig aufeinander wirken. Um diesen Wert leicht zu erlangen, empfiehlt es sich, nach der Konzentration (c) zu differenzieren:

$$d(\eta/\eta_0)/dc = A^c \ln A.$$

Bei der unendlichen Verdünnung, $c = 0$,

$$d(\eta/\eta_0)/dc = \ln A.$$

Daher kann die Viskositätsformel von Polson folgenderweise umgeschrieben werden:

$$1 + \ln A = 1 + 4.0\,V + 0.098\,V\,(b/a)^2. \quad \ldots\ldots\ldots\ldots (4)$$

3) Molekulargewicht und Gestalt.

Herzog, Illig und Kudar[21] und Perrin[22] leiteten theoretisch eine Beziehung zwischen Reibungskonstante und Achsenverhältnis bei der Diffusion ellipsoider Tilchen ab. Für längliche ellipsoide Teilchen gilt

$$\frac{f}{f_0} = \frac{D_0}{D} = \frac{\sqrt{1-\rho^2}}{\rho^{\frac{2}{3}} \ln \frac{1+\sqrt{1-\rho^2}}{\rho}}, \quad \ldots\ldots\ldots\ldots (5)$$

und zwar für abgeplattete ellipsoide Teilchen gilt

$$\frac{f}{f_0} = \frac{D_0}{D} = \frac{\sqrt{\rho^2-1}}{\rho^{\frac{2}{3}} \operatorname{Tan}^{-1}\sqrt{\rho^2-1}}. \quad \ldots\ldots\ldots\ldots (6)$$

Hierin ist $\rho = b/a$, b gleich dem Radius am Äquator und a gleich der halben Länge der Rotationsachse, während f die Reibungs-

konstante des Teilchens und f_0 dieselben des sphärischen Teilchens darstellt, das die gleiche Masse besitzt. f/f_0 bedeutet somit den Abweichungsgrad des Teilchens von der Kugelform, was von Svenberg als die assymmetrische Konstante beziechnet worden ist. Ersetzt den aus G1.(4) erhaltenen Wert von b/a in G1.(5) oder (6), so kann man f/f_0 leicht berechnen.

Zwischen Molekulargewicht, Diffusionskonstante und f/f_0 gibt es eine bequeme Beziehung, die aus Ergebnissen der Ultrazentrifugierungsversuchen von Svedberg[23] abgeleitet wurde, wie folgt:

$$M = \frac{K(f/f_0)^3}{D^3 V}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

$$K = \frac{(RT)^3}{162\,\pi^2 \eta^3 N_2}$$

z.B. bei 10°C, $K = 0.998 \times 10.^{-16}$

Wie obenerwähnt, sind wir nun imstande, das Molekulargewicht irgendeines Proteins aus Diffusions- und Reibungs-daten zu berechnen. Polson erwies die erfolgreiche Anwendbarkeit dieser Methode auf verschiedene Proteine durch Vergleichung mit Ergebnissen aus Ultrazentrifugierungsversuchen von Svedberg.

Aus dem spezifischen Partialvolumen und b/a kann man weiter die absolute Gestalt der als rotierende Ellipsoide angenommenen Proteinmoleküle berechnen. Für das Volumen des rotierenden ellipsoiden Teilchens gilt

$$V_0 = \frac{4}{3}\,\pi a^2 b, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

wobei V_0 das Volumen eines Moleküls in der Lösung bedeutet.

4) Ergebnisse der Berechnungen.

Aus den schon im experimentellen Teil geschilderten Daten über Katalase können die folgenden in obenerwähnter Weise berechnet werden.

$b/a = 3.36$
$f/f_0 = 1.14$ (für das längliche)
$= 1.13$ (für das abgeplattete)
$M = 249{,}900$ (für das längliche)
$= 252{,}800$ (für das abgeplattete)
$a = 3.0 \times 10^{-7}$ cm
$b = 10.1 \times 10^{-7}$ cm

Zusammenfassung

1. Die Diffusionskonstante der Katalase bei 10°C wurde nach der Northropschen Methode bestimmt.

2. Die Reibungskonstante der Kataläselösung und das spezifische Partialvolumen der Katalase wurden ermittelt, woraus nach Polson das Achsenverhältnis des als rotierendes Ellipsoid angenommenen Katalasemoleküls berechnet wurde.

3. Aus Diffusionskonstante und Achsenverhältnis wurde das Molekulargewicht der Katalase berechnet, wie 249,900 für das längliche Teilchen und 252,800 für das abgeplattete.

Ich erlaube mir hiermit dem verstorbenen Herrn Prof. Dr. M. Kitagawa für seine freundliche Anleitung und steten Ratschläge meinen herzlichsten Dank auszusprechen. Ganz besonderen Dank schulde ich auch Herrn Prof. Dr. Y. Oshima und Herrn Prof. Dr. I. Yamasaki für ihre gütigen Beratungen bei der Beschreibung dieses Berichtes.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

STUDIES ON THE PROTEIN OF SOY BEAN

The Effects of Neutral Salts of Low Concentration on the Stability of Soy Bean Protein Dispersed in Water

Masaru Funatsu

Introduction

It is well known that the effects of electrolytes on protein sol show a remarkable difference depending upon their concentration. As has been shown by Graham, the aggregation of protein on flocculation is quite different from that on salting-out.

Especially a protein sol which consists of two or more kinds of protein shows a particular behavior toward the electrolytes. In such a protein sol flocculation of protein takes place in a different manner from a simple protein sol depending upon the proportion of its protein components.[1] As Plötner[2] has already reported, serum shows an inconsistent stability toward sulfosalicylic acid depending upon the proportion of globulin to albumin in serum. Moreover, the proportion of lactalbumin to casein in milk can easily account for the fact that various kinds of milk have different stability to acid or rennin.[3]

Now, on extraction of soy bean meal with distilled water, the greater parts of globulin will dissolve in combination with salt in meal. Thus we obtain a polyphase protein colloid, in which two types of protein, i.e., globulin of lyophobic nature and albumin and proteose of lyophilic nature, disperse in water containing minute quantities of salts derived from soy bean meal. In this

solution proteins are negatively charged by adsorbing the electrolytes derived from soy bean and are in a state of so-called unsaturated colloid practically identical with soil colloid, because, as later mentioned, the quantity of all the metal ions is not enough to saturate the protein particles. The protein sol in the watery extract of soy bean may not be considered pure sol, since various organic substances in soy bean are extracted with distilled water together with the protein.

Therefore, for studying the dispersion-state of protein particles, at first, it must be decided, whether or not we can use the extract as a pure protein sol. Experiment has shown that it is not unreasonable to treat the extract as a pure sol so far as present studies are concerned.

On adding neutral salts to this protein sol, it was observed that flocculation, which occurs due to the unstable colloidal state of globulin, takes place at about 0.04 Mol. concentration of salt in different ways depending upon the proportion of albumin to globulin. This phenomenon can be clearly explained by the protective action of albumin against the flocculation of globulin. This result, furthermore, has been verified by an experiment, in which egg albumin used instead of soy bean albumin produced the same effect on the flocculation. Nevertheless, the mechanism of the protective action of one protein against the other has never been fully explained.

The present paper deals with the discussion of the mechanism from the point of view of hydration under the assumption that the adsorbed albumin plays the same rôle as bound water against the globulin particle.

Experimental Method

Preparation of soy bean protein

Crush the Manchurian yellow soy bean by hammer crusher, avoiding heating as much as possible. After removing the testa by wind, extract the lipoids with ether and further grind it into fine powder by mortor.

Extract the protein of this soy bean meal by shaking with 50 times its weight of distilled water for two hours in room tempera-

ture (24–25°C). Centrifuge the extract (3000 r./min., 30 min.), filter the supernatant of extract under suction through the filterpulp 2 cm. thick. The clear filtrate contained 1.17–1.20 mg nitrogen in c.c. and its pH value was 6.4–6.5.

Estimation of the relative turbidity of sol[4]

The state of dispersion, that is, the state of aggregation of protein particle due to the addition of salt was concluded from its relative turbidity. And the relative turbidity of sol has been estimated by measuring its Tyndall light, since at a constant concentration of protein the strength of Tyndall light of sol is proportional to the volume of particle within a certain range of size of particle. This fact is clearly seen from the formula of Rayleigh:

$$I = k\frac{n v^2}{\lambda^4} = k\frac{c v}{d\lambda^4}$$

The measurement of the strength of Tyndall light has been made by means of Pulflich's Step Photometer (for turbidity) with filter L_2 and milky filter No. 4 for standard light, unless otherwise mentioned.

The values of relative turbidity of sol were represented with the strength of Tyndall light, I. For the convenience of the direct comparison between those of various sol, the proportion of the relative turbidity of each sol to a standard sol without any addition of salt was calculated and expressed as RV.

Measurement of viscosity[5]

The relative viscosity of the sol was determined by using Ostwald's viscosimeter. The viscosity of protein sol does not conform to the formula of Einstein-Smoluchowski: $\eta_s = \eta_0(1 + K\varphi)$ and gives extremely high value. This abnormality in viscosity chiefly depends upon the high hydration of protein particle and has been shown by a value of $(\eta_s - \eta_0)/\eta_0$ according to Kruyt et al.

By the addition of electrolytes the electrical charge of particle changes due to the charge of electrolyte ions and the increase of relative viscosity can be represented by following value $(\eta_{s+e} - \eta_e)/\eta_e$ where, η_{s+e} the viscosity of sol added to the electrolyte and η_e the viscosity of dispersed medium after the addition of salt.

Experimental Results and Discussion

I. Can soy bean protein sol be used interchangeably with the pure protein sol?

The soy bean protein sol contains various organic substances derived from soy bean meal. It is considered that the organic substances besides protein produce more or less changes on the properties of protein sol.

If the extent of the influence of other organic substance besides protein is not great as to change the colloidal behavior of protein particle or to change the relationship between the values of each components of Rayleigh's formula, we may deal with the pure one. By these means, it must be decided whether or not the value for $I\lambda^4$ of these sols, which have equal concentration of protein, may be maintained always constant to various wave lengths of light, as is required by the formula of Rayleigh. Table 1 clearly shows the values for $I\lambda^4$ remain constant irrespective of the wave length of light. Hence, it follows that protein sol behaves just like a pure protein sol in spite of the co-existance of other organic substances.

Table 1. $I\lambda^4$ value of soy bean protein sol (at 25°C).

Filter	Wave length of maximum filtration λ mμ	Soybean protein sol nitrogen content 1.21 mg/cc. pH=6.5		Soybean protein sol nitrogen content 0.61 mg/cc. pH=6.5		Soybean protein sol nitrogen content 1.21 mg/cc. pH=6.4	
		I	$I\lambda^4.10^{-13}$	I	$I\lambda^4.10^{-13}$	I	$I\lambda^4.10^{-13}$
L_1	590	270.3	3.3	208.3	2.5	285.7	3.5
S_{57}	575	344.8	3.7	232.6	2.5	370.4	3.9
S_{53}	531	416.7	3.3	312.5	2.4	444.4	3.6
S_{50}	494	490.2	2.4	416.7	2.0	666.7	3.2
S_{47}	465	500.0	2.3	434.8	2.0	645.2	3.0
S_{43}	438	—	—	487.8	1.8	952.4	3.5
Mean value of $I\lambda^4.10^{-13}$			3.2		2.3		3.5

II. Flocculation of soy bean protion by the addition of salt.

By the addition of the electrolytes, such as ammonium sulfate, sodium sulfate, sodium chloride, to the soy bean protein sol, a flocculation appears at a certain concentration of salt lower than that needed for salting out. Between these two concentrations of salt, peptization takes place and the protein particles disperse in the high degree.

Table 2. Effect of salts to soy bean protein sol and soy bean albumin sol.

Conc. of salt \ Relative turbidity	Soybean protein sol nitrogen content 0.235~0.245 mg/cc. pH=6.4								Soybean albumin sol nitrogen content 0.03 mg/cc. pH=6.4					
	$(NH_4)_2SO_4$		Na_2SO_4		$MgSO_4$		NaCl		$(NH_4)_2SO_4$		Na_2SO_4		NaCl	
	I	RV	I	RV	I	RV	I	RV	I	RV	I	RV	I	RV
0.000	78	100	111	100	111	100	90	100	53	100	53	100	53	100
0.001	79	101	111	100	571	514	90	100	52	98	51	96	52	98
0.002	86	110	115	103	flocculation		91	101	51	97	49	92	52	98
0.004	100	128	127	114	flocculation		91	101	50	97	47	89	50	94
0.010	222	284	164	148	flocculation		105	117	50	97	31	59	39	74
0.020	1111	1422	435	391	flocculation		161	179	50	97	27	51	37	70
0.040	1428	1828	1000	900	flocculation		588	653	42	79	28	53	34	64
0.100	909	1164	833	750	1428	1285	1667	1852	30	54	29	55	37	70
0.200	124	159	233	209	222	200	1250	1389	24	45	31	59	39	74
0.400	100	128	187	186	156	140	455	505	18	34	34	64	42	79
1.000	192	246	400	360	139	125	167	185	18	34	50	94	47	89

To 10 cc of soy bean protein sol prepared by the method previously mentioned add varying volumes of 2 to 0.002 Mol. solution of neutral salts, and increase the total volume to 50 cc with distilled water. The protein sols having 0.001 to 1.0 Mol. concentration of salt are then obtained.

Their turbidities are measured by means of Pulfrich's Step Photometer (for turbidity) in 30 min. after adding salt. The results obtained are shown in Table 2 and illustrated in Fig. 1.

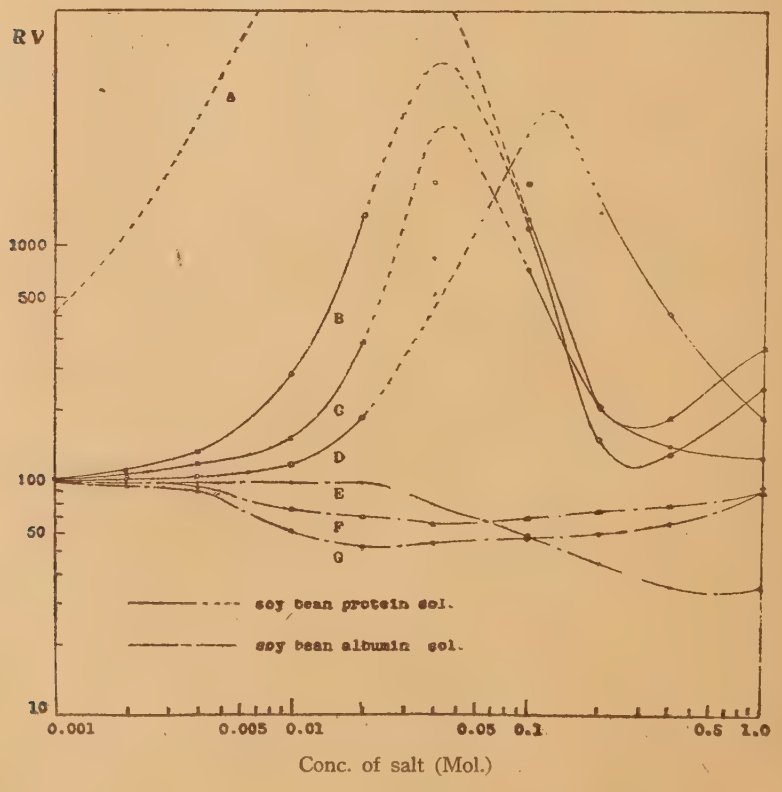

Fig. 1. Effect of salt to soy bean protein sol and albumin.

A: $MgSO_4$ B: $(NH_4)_2SO_4$ C: Na_2SO_4 D: NaCl
E: $(NH_4)_2SO_4$ F: NaCl G: Na_2SO_4

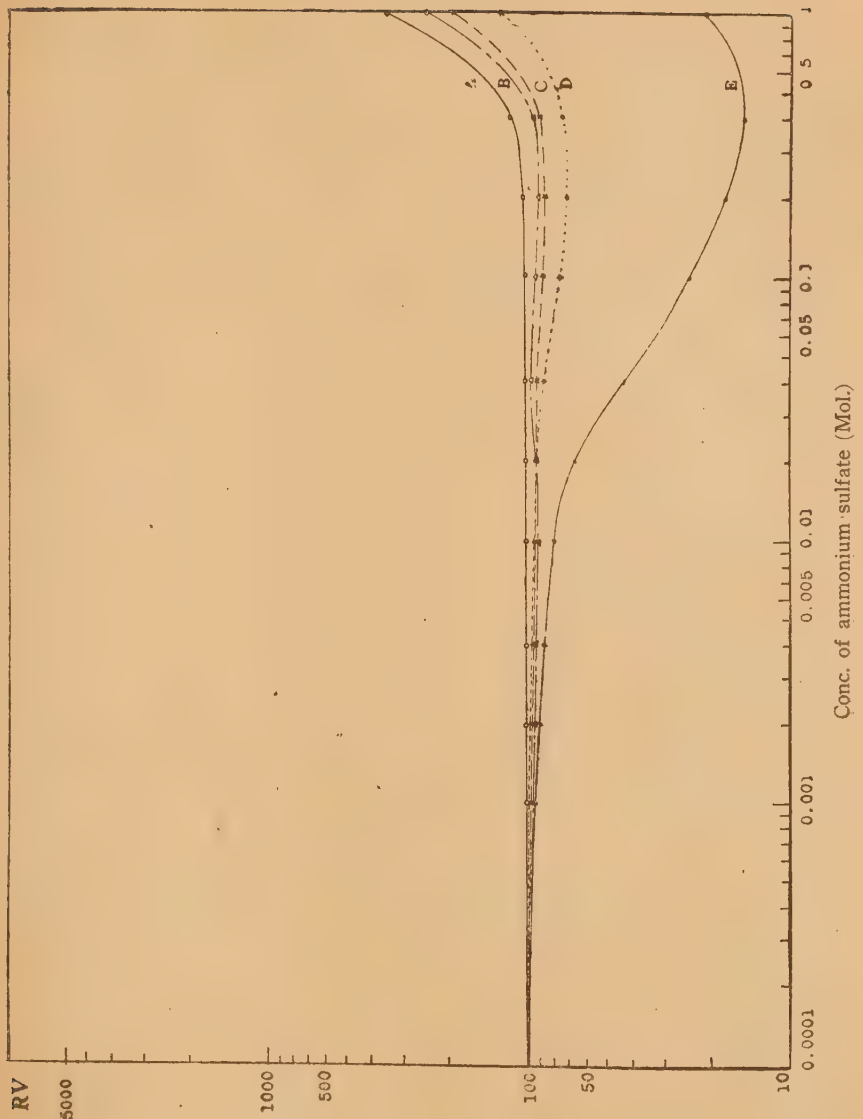

Fig. 2. Effect of NaCl.

A: 10% NaCl B: 5% NaCl C: 4% NaCl D: 2.5% NaCl E: 2%NaCl

It will be noticed that the gradual increase in the relative turbidity after the addition of salt is followed with the abrupt increase to a maximum, giving rise to flocculation. Then the rapid peptization takes place, followed with salting out.

In the case of adding ammonium sulfate, for instance, the relative turbidity of sol increases gradually until 0.01 Mol. concentration of salt and reaches its maximum value at about 0.04 Mol. Salting out takes place in concentration greater than 0.4 Mol. These turbidity curves are given in similar way by various salts, but, of course, the point of flocculation varies with the kinds of salt.

The flocculation occurs also in 10 to 20 times diluted sol (see Table 5). It seemes to indicate that the concentration of electrolytes originally contained in the protein sol is not inherent to the flocculation. On the other hand, the series of protein sol, to which NaCl has been previously added up to 2.5%, 5% and 10% respectively, give no flocculation by adding a solution of ammonium sulfate which containes the respective concentration of NaCl. (see Fig. 2). It is considered that by adding NaCl the unsaturated protein particle adsorbs Na ion and changes into saturated colloid, thus resulting in the increase of bound-water to maximum value and dispersal in stable state. Even if the substitution of ion between Na and NH_4 takes place by adding ammonium sulfate, the protein particle will be kept in stable state and give no flocculation.

From these two experiments above mentioned, it will be seen that the greater parts of the protein particles are in unsaturated state. The fact that albumin does not show flocculation by adding such a small quantity of salt, makes us conclude that this flocculation takes place chiefly due to unstable dispersion state of globulin. Even though globulin gets into solution by combining with the inorganic salts in soy bean and increases its stability in solution by combining with albumin, yet it exists in relatively unstable state, i.e., in unsaturated colloidal state. This unstable globulin is further protected by this combined albumin from the action of salt, that is, from the attack of salt to that governing the stability of globulin.

The addition of salt results in the breakdown of the electrical potential as well as the decrease of bound-water, which cause the lowering of the stability of protein particles, especially of globulin.

But at the same time there is a resistance to the change of stability, which is given by albumin.

The change of the electrical condition of protein particle, due to the substitution of adsorbed ion, the change of Donnan potential and the change of ionic activity, can be demonstrated by the change in hydration as well as the change in viscosity of sol.[6] The flocculation, therefore, will be explained more in detail by measuring the viscosity of sol[7].

The change in viscosity by the addition of ammonium sulfate has been shown by the ratio, $(\eta_{s+e}-\eta_e)/\eta_e$, in Table 3 and Fig. 3.

Table 3. Change of viscosity and that of pH of soy bean protein sol by adding ammonium sulfate.

Concentration of salt Mol./L	I	pH	η_s/η_w	η_{s+e}/η_e	$\frac{\eta_{s+e}/\eta_e}{\eta_e/\eta_w}\cdot 100$	$\frac{\eta_s-\eta_w}{\eta_w}$	$\frac{\eta_{s+e}-\eta_e}{\eta_e}$	
0.000	78	6.40	1.0069		100.0	0.0069		100
0.001	79	6.72		1.0197	101.3		0.0197	281
0.002	86	6.72		1.0245	101.7		0,0245	350
0.004	100	7.03		1.0250	101.8		0.0250	357
0.010	222	7.03		1.0257	101.9		0.0257	367
0.020	1111	7.04		1.0254	101.8		0.0254	363
0.040	1428	7.05		1.2331	122.5		0.2331	3329
0.100	909	6.98		1.0130	100.6		0.0130	186
0.200	124	6.98		1.0102	100.3		0.0102	146
0.400	100	6.98		1.0119	100.5		0.0119	170
1.000	192	6.98		1.0118	101.1		0.0178	254
1.200	—	6.98		1.0232	101.6		0.0232	331

On adding ammonium sulfate, the relative viscosity of sol hardly increases until the concentration of salt reaches to 0.04 Mol., except for the initial little increase, but at the point of flocculation it increases suddenly to a maximum high value and then falls down giving rise to peptization. A salting out will follow with a gradual increase in the viscosity. The change of pH of sol parallels the change of viscosity, but not in such great magnitude.

The initial increase in viscosity is caused by the rapid adsorption of ammonium ion by unsaturated protein particle. At the same time, the ionic substitution will occur between adsorbed and added ions. Inherently the soy bean protein particle seems to be similar to K-colloid, judging from the quantity of electrolytes of soy bean. The adsorption power of ammonium ion by protein

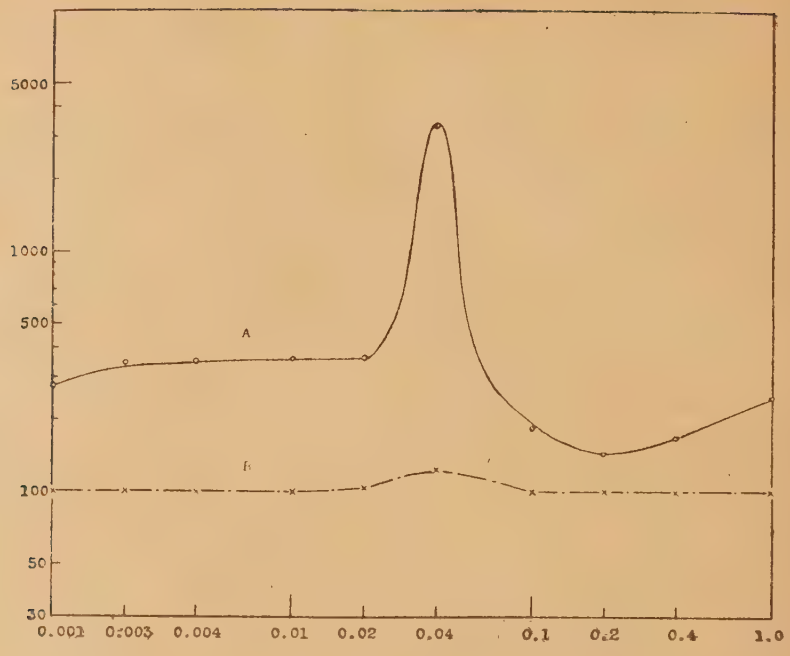

Fig. 3. Change in viscosity.

$$\text{A}: \frac{\eta_{s+e}-\eta_e}{\eta_e}\cdot 100 \qquad \text{B}: \frac{\frac{\eta_{s+e}}{\eta_e}}{\frac{\eta_s}{\eta_w}}\cdot 100$$

particle is so much stronger than that of potassium that the ionic substitution occurs between them. This phenomenon may be continued to the end of ionic substitution and until finally the colloid becomes saturated with ammonium ion. Since the hydration of ammonium ion is smaller than that of potassium ion, the decrease of hydration of protein particle must accompany the substitution of ion.[8] The changing into saturated colloid, will results in the depression of Donnan potential and ionic activity which causes the decrease of total potential of protein micelle. But all of those tendencies to lower the hydration of particle appeared to be negligible compared to the increase of hydration by adsorbed albumin. In this connection the combination of albumin means the increase of bound-water. Nevertheless, owing to the change of those electrical conditions,[9] the total potential of micelle becomes lower.

The bound-water and combined albumin separate gradually and partially from globulin. Some of those globulin particles which repel each other adsorb together, resulting in the lowering of electrical resistance. And so a large particle is adsorbed by a smaller one. Thus the increase in viscosity due to the enlargement of volume of particle by aggregation will counter balance the decrease in viscosity caused by the depression of hydration of particle.

In this way the viscosity of sol appears to remain constant. But at 0.04 Mol. concentration of ammonium sulfate increases the viscosity rapidly and the flocculation occurs. This will be accounted for by the breakdown of the combination of albumin and globulin. In consequence, parts of globulin are subjected to the attack of salt. This leads to the rapid flocculation of globulin particle. Then the viscosity falls down very rapidly and this means so-called peptization. At this point the potential of particle falls down below the critical potential and albumin will separate completely from globulin. Over this concentration of salt the combination of globulin with albumin will be completely destroyed and globulin will become sensitive to salts. Adding more quantities of salt increases the viscosity. This will mean slow aggregation.

Because of this action, we conclude that albumin plays an important rôle in flocculation and that globulin will become just as hydrated. It will be presumed, accordingly, that the flocculation will take place in a different manner depending upon the proportion of albumin to globulin. To ascertain this assumption, the following experiment has been carried out.

III. The influence of the proportion of albumin to globulin upon the effects of salt.

To make clear the influence of the ratio between globulin and albumin upon the effect of salt, soy bean albumin was added to the soy bean protein sol in different concentrations and the effect of ammonium sulfate upon them has been observed. The soy bean albumin was prepared by the following method. The soy bean protein sol has been subjected to dialysis for 2 days against water using collodion membrane. The precipitated globulin was separated by centrifuge and supernatant albumin fraction has further been

Table 4. Influence of ratio $\frac{\text{globulin}}{\text{albumin}}$ upon the effect of ammonium sulfate.

Number of sol	I		II		III		IV		V		VI	
The composition of sol	Soybean protein sol + albumin fraction		Soybean protein sol + albumin fraction		Soybean protein sol + albumin fraction		Soybean protein sol + albumin fraction		Soybean protein sol (original)		Soybean protein sol (10 times diluted	
Nitrogen mg/cc	0.021		0.041		0.085		0.182		0.285		0.028	
Ratio, $\frac{\text{globulin}}{\text{albumin}}$	0.02		1.83		5.12		10.51		13.83		13.83	
Conc. of salt (Mol.) \ Relative turbidity	I	RV	I	RV	I	RV	I	RV	I	RV	I	RV
0.000	89	100	87	100	80	100	82	100	78	100	15	100
0.001	87	97.7	85	97.7	65	81.3	85	103.7	79	101.1	15	100
0.002	86	96.6	77	88.5	59	73.8	86	104.9	86	110.0	15	100
0.004	86	96.6	32	36.8	48	60.0	94	114.7	100	128.0	15.5	103.4
0.010	86	96.6	29	33.3	57	71.3	143	174.2	222	284.2	20	133.4
0.020	86	96.6	80	91.9	263	329.0	556	677.8	1111	1422.1	77	513.6
0.040	70	78.6	250	287.3	667	833.4	1250	1525.0	1428	1827.8	263.2	1755.6
0.100	45	50.5	189	216.8	435	543.5	833	1016.6	909	1163.5	169.5	1130.6
0.200	15	16.8	32	36.8	69	86.3	164	226.8	124	158.7	34	226.8
0.400	12	13.5	29	33.5	57	71.3	122	140.1	100	128.0	21	140.0
1.000	30	33.7	45	51.7	93	116.3	183	220.1	192	245.8	33	220.1

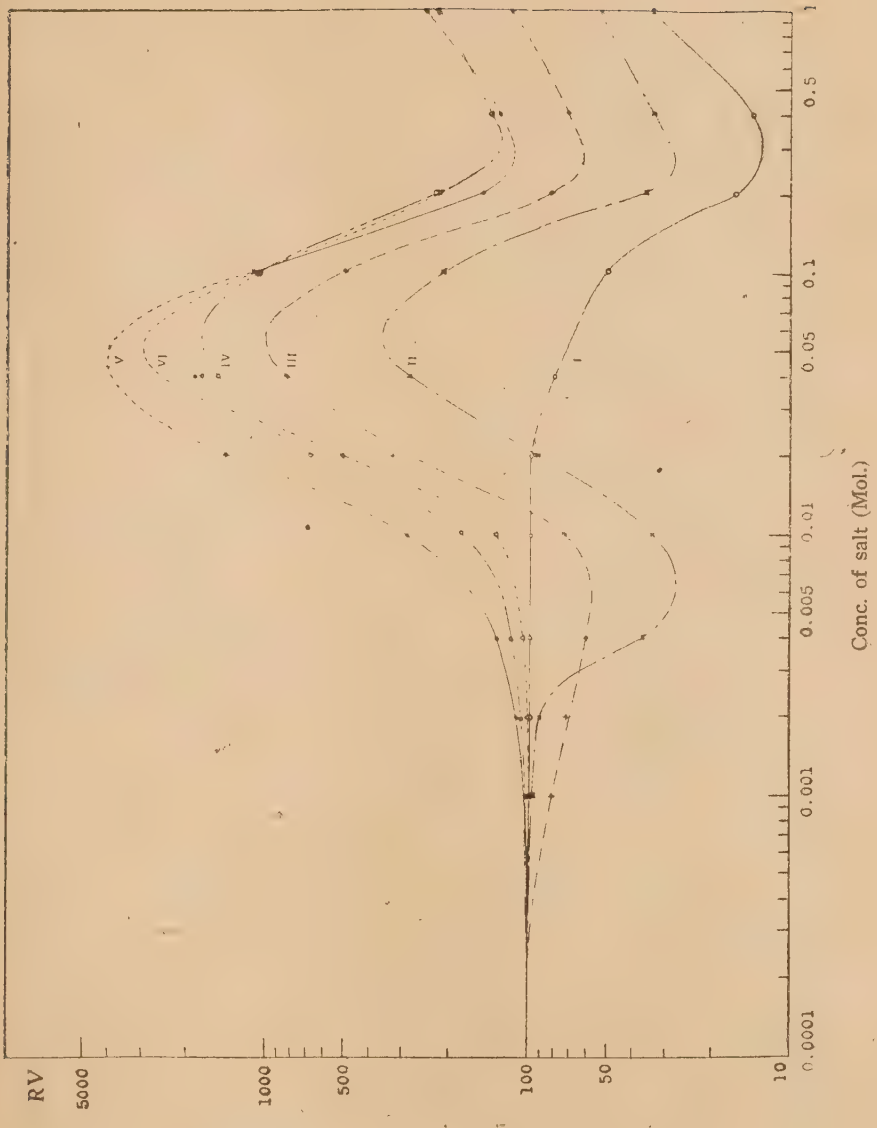

Fig. 4. Influence of globulin/albumin ratio upon the effect of ammonium sulfate to soy bean protein sol.

subjected to dialysis for 2 days against distilled water. The precipitated globulin was again centrifuged off and clear albumin fraction was obtained by filtration through filter pulp.

The determination of the ratio between globulin and albumin was made by the method by Muramatsu.[10]

Experimental results were given in Table 4 and Fig. 4.

The flocculation occurs in a different manner on changing the proportion of albumin to globulin. From the fact* that there is no change of colloidal state in diluted sol due to adding albumin fraction, it will be clear that the decrease of nitrogen content gives no effect upon the flocculation. This phenomenon may be accounted for by the increase of stability of globulin due to the protective action of albumin. The following data will show clearly the intimate relation between RV and the proportion of globulin to albumin at 0.04 Mol. ammonium sulfate, that is, in a point at which the dispersity of protein particles is minimum.

$\frac{\text{globulin}}{\text{albumin}}$ ratio	RV
0.02	78.6
1.83	287.3
5.12	833.4
10.51	1525.0
13.85	1827.8

As the ratio changes, there also occurs the flocculation in a different manner. When the ratio falls down to 0.02, almost no flocculation takes place. The above result apparently reveals the important rôle of albumin in the flocculation. In this connection it will naturally be expected that, in place of soy bean albumin, egg albumin will have the same effect on the flocculation. Practically the same results were obtained with egg albumin, as is shown in Table 5 and Fig. 5. Egg albumin behaves in a manner to protect soy bean protein from the influence of salt.

In conclusion, it is a remarkable fact regarding the protective action of protein, that serum albumin as well as egg albumin

* That the dilution due to the addition of albumin fraction gives no influence upon the effect of ammonium sulfate, was ascertained by examining the effect of salt on the 10 or 20 times diluted soy bean protein sol. See Tab. 4 & 5.

Table 5. Effect of egg albumin on the flocculation of soy bean protein by the addition of sodium sulfate.

Number of sol	I		II		III		IV		V		VI		VII	
The composition of sol	Egg albumine sol		Soybean protein sol + egg albumin sol		Soybean protein sol + egg albumin sol		Soybean protein sol + egg albumin sol		Soybean protein sol		Soybean protein sol (10 times diluted)		Soybean protein sol (20 times diluted)	
Nitrogen, mg/cc.	0.142		0.146		0.162		0.188		0.285		0.029		0.014	
Ratio, $\frac{\text{globulin}}{\text{albumin}}$	0.00		0.09		0.34		1.24		13.83		13.83		13.83	
Conc. of salt (Mol.) \ Relative turbidity	I	RV	I	RV	I	RV	I	RV	I	RV	I	RV	I	RV
0.000	66	100.0	112	100.0	106	100	100	100	89	100	21	100	15	100
0.001	65	98.5	111	99.4	104	97.9	98	98	94	105.6	21	100	15	100
0.002	65	98.5	106	95.7	102	96.0	95	95	100	112.3	21	100	15	100
0.004	64	97.0	104	93.7	99	93.0	94	94	102	114.5	22	105	15	100
0.010	63	95.4	100	89.9	97	92.0	104	104	122	137.0	24	114	17	113
0.020	62	94.0	114	102.2	189	177.3	238	238	333	374.3	70	333	50	330
0.040	55	83.3	149	134.2	476	447.5	625	625	769	863.8	179	851	127	844
0.100	52	78.8	128	115.3	294	276.4	434	434	526	591.0	143	680	101	673
0.200	54	81.8	58	52.2	78	73.3	110	110	149	167.6	31	148	21	140
0.400	59	89.4	55	49.5	76	71.4	104	104	149	167.6	35	167	23	153
1.000	74	112.1	91	81.8	145	136.2	265	265	455	510.4	62	295	47	313

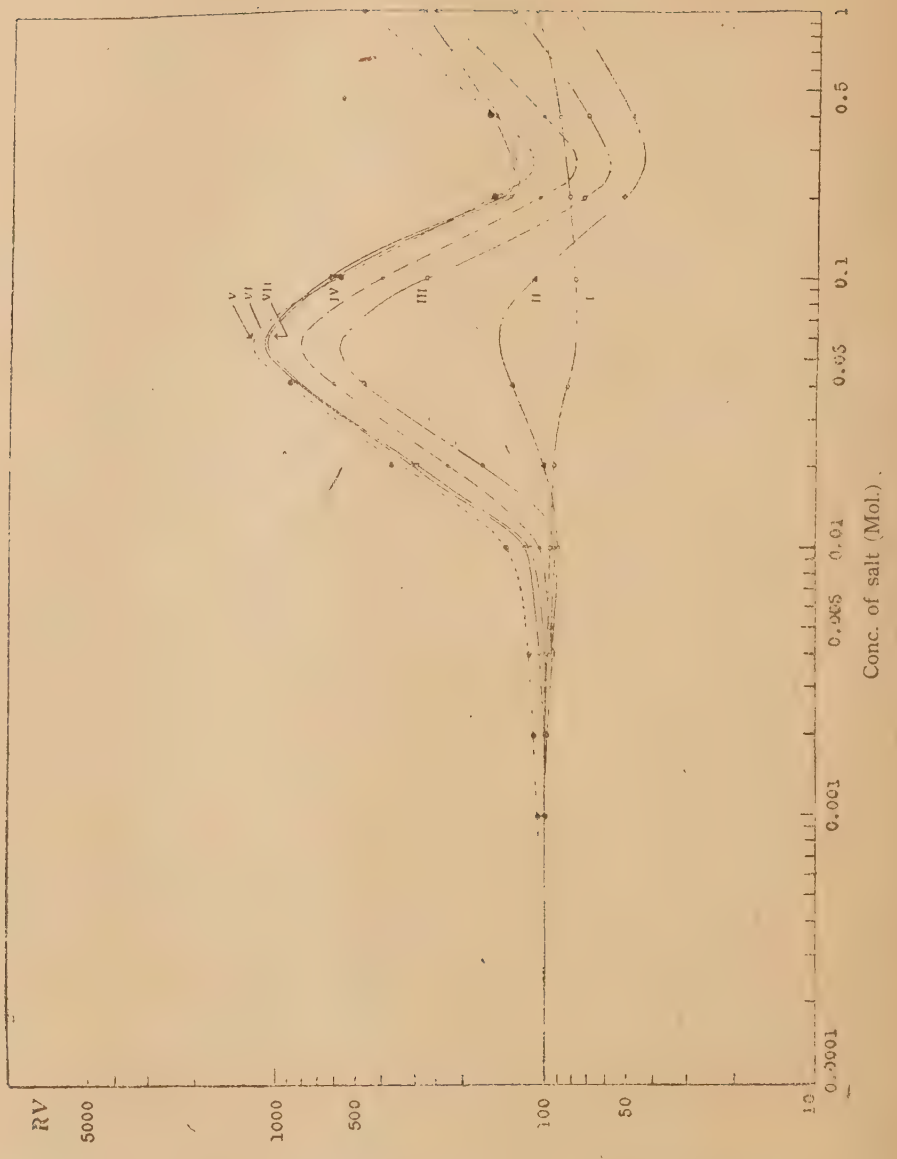

Fig. 5. Effect of egg-albumin to the flocculation of soy bean protein sol by Na_2SO_4.

will be split by the action of clupein,[11] and also that in serum the desaggregation of globulin particle will take place by the action of albumin, as has been reported by McFarlane.[12] This breaking of the larger protein molecule by the action of smaller one may have an important rôle in protective action. In other words, the protective action may take place by the desaggregation of large protein molecule, thus bringing the unstable state of large molecule into the stable one. It is not yet clear whether the protective action of albumin is due to the combination with globulin or due to the desaggregation of globulin.

Summary

On adding neutral salt, such as ammonium sulfate, sodium sulfate and sodium chloride, to the soy bean protein sol, flocculation occurs in about 0.04 Mol. concentration of salt. It seems that this phenomenon is caused chiefly by globulin which is dispersed in relatively unstable state. Albumin does not flocculate at such a concentration, but yet it plays an important rôle in protecting globulin against the flocculation. The flocculation occurs in a different manner depending on varying proportion of albumin to gobulin. This will be explained by the protective action of albumin against the flocculution of globulin. In this connection egg-albumin has also the same effect upon the flocculation. The mechanism of this protective action may be postulated as follows: the adsorbed albumin plays the same rôle as bound-water, that is, albumin combines with globulin and protects it from the action of salt in such a way as to give the protein micelle greater hydration, higher potential and more stability against salt.

In conclusion, the author wishes to express his hearty thanks to Dr. Seiichi Izume at Central Laboratory of S.M.R., Professor Yasuyoshi Oshima and Professor Tetsuo Tomiyama for their helpful advice.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 9, No. 2
May 31, 1949

A MODIFICATION OF EKDAHL'S METHOD FOR ROUTING FLOODS THROUGH RESERVOIRS

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1.

In a previous paper[1] we suggested a nomographic solution of Ekdahl's equation in the problem of reservoir storage. Later it came to our notice that the idea had been anticipated by Prof. Chesley J. Posey, who had proposed a slide-rule solution, referring to the construction of an alignment chart as an alternative procedure.[2] The question naturally arises: Is it possible to construct a similar slide-rule based on any other formula than Ekdahl's? The answer is in the affirmative, though the statement must be made with a mental reservation. In the present note we shall show that, by using a parabolic formula of integration instead of the trapezoidal, we can set up an analogue to Ekdahl's equation, which permits the same treatment as the latter.

2.

Let the equation of continuity be

$$\frac{dV}{dt} = Q(t) - q(V), \tag{1}$$

where t denotes the time, V the storage, $Q(t)$ the rate of inflow, $q(V)$ the rate of outflow.

Then

$$V_2 - V_1 = \int_{t_1}^{t_2} Q(t)\,dt - \int_{t_1}^{t_2} q(V)\,dt, \tag{2}$$

where the subscripts 1 and 2 refer to the beginning and end of the time interval respectively. If in the evaluation of the second integral in (2), we approximate $q(V)$ by a polynomial of degree $m+n+1$ in t, making m-th order contact with $q(V)$ at $t=t_1$ and n-th order contact at $t=t_2$, we get an algebraic or a transcendental equation, from which, given the value of V_1, V_2 may be determined. Thus if we take $m=0$, $n=0$, we have the Ekdahl formula:

$$V_2+\frac{h}{2}q(V_2) = V_1-\frac{h}{2}q(V_1)+\int_{t_1}^{t_2} Q(t)\,dt, \tag{3}$$

h representing t_2-t_1.

If we take $m=1$, $n=0$, we obtain

$$V_2+\frac{h}{3}q(V_2) = V_1-\frac{2}{3}hq(V_1)+\frac{h^2}{6}q'(V_1)q(V_1)-\frac{h^2}{6}q'(V_1)Q(t_1) + \int_{t_1}^{t_2} Q(t)\,dt. \tag{4}$$

For let $q(V)$ in (1) be approximated by a polynomial of the second degree in t:

$$p(t) = a_0+a_1t+a_2t^2,$$

were a_0, a_1, a_2, are undetermined coefficients.

The conditions to be fulfilled by $p(t)$ are:

$$q(V_1) = p(t_1)=a_0+a_1t_1+a_2t_1^2,$$
$$q'(V_1)(dV/dt)_1 = p'(t_1) = a_1+2a_2t_1,$$
$$q(V_2) = p(t_2) = a_0+a_1t_2+a_2t_2^2,$$

where $(dV/dt)_1$ denotes the value of dV/dt at $t=t_1$.

Hence

$$q(V_2)-q(V_1) = a_1(t_2-t_1)+a_2(t_2^2-t_1^2),$$

or

$$\frac{q(V_2)-q(V_1)}{t_2-t_1} = a_1+a_2(t_2+t_1).$$

By subtraction we get

$$\frac{q(V_2)-q(V_1)}{t_2-t_1}-q'(V_1)(dV/dt)_1 = a_2(t_2-t_1).$$

On the other hand, $p'(t_2)-p'(t_1) = 2a_2(t_2-t_1)$.

Eliminating a_2 between the last two equations, we obtain

$$p'(t_2)-p'(t_1)=2\left\{\frac{q(V_2)-q(V_1)}{t_2-t_1}-q'(V_1)(dV/dt)_1\right\}.$$

Hence, having regard to equation (1), we find by the Euler-Maclaurin formula

$$\int_{t_1}^{t_2}q(V)dt-\int_{t_1}^{t_2}p(t)dt=\frac{t_2-t_1}{2}\left\{p(t_2)+p(t_1)\right\}-\frac{(t_2-t_1)^2}{12}\left\{p'(t_2)-p'(t_1)\right\}$$

$$=\frac{t_2-t_1}{3}\left\{2q(V_1)+q(V_2)\right\}+\frac{(t_2-t_1)^2}{6}q'(V_1)\left\{Q(t_1)-q(V_1)\right\}.$$ *

Substitution of this expression for the second integral in (2) yields formula (4).

Now the reason why (3) is adapted for numerical calculation is that it is of the form:

$$A(V_2)=B+C(V_1),$$

in which $A(V)$ and $C(V)$ can be represented graphically, once the value of h is fixed; and B is a quantity whose values are computable beforehand. It is these properties of (3) that enable us to solve it conveniently with the aid of numerical tables, or graphs, or a slide-rule, or alternatively, a nomogram with three parallel straight supports.

Turning to equation (4), we see it also assumes a similar form:

$$A'(V_2)=B'+C'(V_1),$$

where B' stands for $-h^2q'(V_1)Q(t_1)/6+\int_{t_1}^{t_2}Q(t)dt$. Here $A'(V)$, $C'(V)$ are of the same nature as $A(V)$, $C(V)$, but B' is determined only after the value of V_1 is found. This difference between B and B', however, does not preclude us from applying to (4) the various devices enumerated above.

We shall illustrate the use of equation (4) by a numerical example.

3.

Suppose a stream issues from a reservoir which is fed by torrential waters. Assuming that the inflow rate varies linearly with the time (t), and the outflow rate as the square of the head

* The remainder term in this formula is found to be $-(t_2-t_1)^4(d^3q(V)/dt^3)_\xi/72$ $t_1<\xi<t_2$.

(z), and that the surface area (F) of the reservoir remains constant, the equation of continuity may be written:

$$F\frac{dz}{dt}=at+b-kz^2, \qquad (5)$$

where a, b, k are constants. This equation is integrable in terms of modified Bessel functions of orders $\pm 1/3$ and their derivatives.

Expanding these functions in power series, we get the general integral:

$$z=\left\{C\left(\frac{1}{\alpha k}+\frac{\alpha}{3}\tau^3+\frac{\alpha^3 k}{3\cdot4\cdot6}\tau^6+\frac{\alpha^5 k^2}{3\cdot4\cdot6\cdot7\cdot9}\tau^9+\frac{\alpha^7 k^3}{3\cdot4\cdot6\cdot7\cdot9\cdot10\cdot12}\tau^{12}+\cdots\right)\right.$$
$$\left.+\frac{\alpha}{2}\tau^2+\frac{\alpha^3 k}{2\cdot3\cdot5}\tau^5+\frac{\alpha^5 k^2}{2\cdot3\cdot5\cdot6\cdot8}\tau^8+\frac{\alpha^7 k^3}{2\cdot3\cdot5\cdot6\cdot8\cdot9\cdot11}\tau^{11}+\cdots\right)\Big\}\Big/\Big\{C\tau\Big(1+\frac{\alpha^2 k}{3\cdot4}\tau^3$$
$$+\frac{\alpha^4 k^2}{3\cdot4\cdot6\cdot7}\tau^6+\frac{\alpha^6 k^3}{3\cdot4\cdot6\cdot7\cdot9\cdot10}\tau^9+\frac{\alpha^8 k^4}{3\cdot4\cdot6\cdot7\cdot9\cdot10\cdot12\cdot13}\tau^{12}+\cdots\Big)$$
$$+1+\frac{\alpha^2 k}{2\cdot3}\tau^3+\frac{\alpha^4 k^2}{2\cdot3\cdot5\cdot6}\tau^6+\frac{\alpha^6 k^3}{2\cdot3\cdot5\cdot6\cdot8\cdot9}\tau^9+\frac{\alpha^8 k^4}{2\cdot3\cdot5\cdot6\cdot8\cdot9\cdot11\cdot12}\tau^{12}+\cdots\Big\}, \qquad (6)$$

where $\tau=at+b$, $\alpha=1/Fa$, and C denotes the integration constant.

Suppose a flood flow sets in, the rate of inflow increasing from zero at $t=0$ to a peak of 240 m^3/sec at $t=12$ (in hours), and thence decreasing to zero at $t=28$. Measuring z in meters and taking $k=50$ m/sec, $F=5\times10^6$ m^2, we may write equation (5) thus:

$$\frac{5\times10^6}{3600}\frac{dz}{dt}=at+b-50z^2, \qquad (5')$$

where $a=20$, $b=0$ for $0\leqq t\leqq12$; $a=-15$, $b=420$ for $12\leqq t\leqq28$.

The factor 1/3600 is introduced to express the rate of change of the head in m/sec, so that in applying formula (6) we have to adopt $5\times10^6/3600$ as the value of F.

If we assume $z=0$ when $t=0$, we have $C=0$ for $0\leqq t\leqq12$. Hence z works out at 0.953259 when $t=12$. From this second condition we get $C=-0.0030158$ for $12\leqq t\leqq28$.

To adapt formula (4) to our problem, we change the dependent variable from V to z by the relation $dV=f(z)dz$, where $f(z)$ denotes the surface area of the reservoir. Then equation (4) in the modified form reads

$$V(z_2)+\frac{h}{3}q(z_2) = V(z_1)-\frac{2}{3}hq(z_1)+\frac{h^2}{6}\frac{q'(z_1)}{f(z_1)}q(z_1)-\frac{h^2}{6}\frac{q'(z_1)}{f(z_1)}Q(t_1)$$
$$+\int_{t_1}^{t_2}Q(t)dt \qquad (4')$$

In our example $V(z)=(5\times 10^6/3600)z$, $Q(t)=at+b$, $q(z)=50z^2$, $q'(z)=100z$, so that, if we take $h=4$, (4′) becomes

$$z_2+0.048z_2^2 = 0.00072\int_{t_1}^{t_2}(at+b)dt+z_1-0.096z_1^2$$
$$-0.00013824z_1(at_1+b-50z_1^2). \qquad (4'')$$

We reproduce in the second column of the accompanying table the successive values of z found by solving this equation repeatedly. The computations have been carried out with the help of the graphs of $z+0.048z^2$ and $z-0.096z^2$.

t	z from (6)	z from (4″)	z from (3)
0	0	0	0
4	0.115	0.115	0.114
8	0.458	0.449	0.444
12	0.953	0.953	0.942
16	1.356	1.357	1.351
20	1.488	1.488	1.492
24	1.435	1.435	1.441
28	1.257	1.256	1.262

The values of z thus computed agree, with one exception, satisfactorily with the corresponding figures in the first column, which represent the values of z as calculated from (6). For comparison the figures obtained by Ekdahl's method with the same value of h are given in the third column. In this case, too, the calculations have been carried out semi-graphically.

As a general rule, our method is superior to Ekdahl's in point of accuracy, for which advantage we pay the price of performing the intermediate computation of $-h^2q'(z_1)Q(t_1)/6f(z_1)$. Whether or not this price is reasonable is a question with which we are not concerned here.

References

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九州大學農學部紀要
第9卷, 第2冊

昭和二十四年五月二十五日印刷
昭和二十四年五月三十一日發行

編輯兼發行者　九州大學農學部
九州大學內

印刷者　堀川庄三

印刷所　九州大學印刷所